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STUDIES ON BACTERIAL CANKER OF TOMATO [1]

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INTRODUCTION

Bacterial canker of tomatoes was first described by E. F. Smith (*24*)[2] in 1909. He cultured the causal organism, which he named *Aplanobacter michiganense*, proved its pathogenicity, and made some cultural studies of the organism and histological studies of diseased plants (*25*, *26*). He called the disease Grand Rapids disease of tomatoes, from the location where it was first found, but in his textbook (*26*) he renamed it bacterial canker of tomatoes. Doidge (*11*) in the same year used the name tomato canker for another disease occurring in South Africa. Gardner and Kendrick (*14*) have identified this South African disease with the bacterial spot of tomato and pepper occurring in the United States, caused by *Bacterium vesicatorium;* and the name "bacterial spot" used by them is commonly accepted for this disease in the United States. Kotte (*16*) suggests that bacterial wilt would be a better name than bacterial canker, since the cracks on the stems are not true cankers; but the existence of another bacterial wilt, caused by *Bact. solanacearum*, and the identification of a cankerlike fruit spot with the disease caused by *A. michiganense* make this name undesirable. It seems justifiable, therefore, to retain the name bacterial canker for the disease caused by *A. michiganense*.

A circular and several notes on bacterial canker have been published by the writer (*4*, *5*, *6*, *7*, *8*). The present paper gives in more detail the results of recent studies.

GEOGRAPHICAL DISTRIBUTION

Bacterial canker is a serious menace to both field and hothouse tomatoes. First described from Michigan in 1909, the disease was later reported from New York, Massachusetts, Pennsylvania, Connecticut, New Jersey, Ohio, and Illinois; but until 1927 it was not known outside of this northern group of States. A severe outbreak occurred in large commercial fields in New York in 1926 (*15*). In 1927 the disease was reported from Georgia, Utah,[3] and Montana; in 1928, from Washington (*21*, *p. 23*), California, and Wisconsin; and in 1929 it appeared in Indiana, Mississippi, and Maryland, and more

[1] Received for publication June 30, 1930; issued December, 1930.

[2] Reference is made by number (italic) to Literature Cited, p. 850.

[3] LINFORD, M. B. PLANT DISEASES IN UTAH IN 1927. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 59: 100-101. 1928. [Mimeographed.]

widely than before in California (*20*). Since the fruit spot caused by *Aplanobacter michiganense* was observed as early as 1917 but was not identified with this disease and was later confused with the bacterial spot caused by *Bacterium vesicatorium*, the distribution is probably wider than here recorded. Certainly Iowa must be included on the evidence of a photograph by Pritchard (unpublished) of typical spotted fruit from Muscatine, Iowa, in 1917.

Bacterial canker has been reported from several foreign countries. Peglion (*22*) in 1915 reported a disease occurring in Italy which he considered identical with bacterial canker. McLarty (*18, 19*) noted its occurrence in British Columbia in 1925 and 1926. In 1928 Bisby and Conners (*2*) found it in Manitoba. Its first appearance in Germany has just been reported by Kotte (*16*). A disease said to resemble this disease more than the one caused by *Bacterium solanacearum* was reported as occurring in an Australian greenhouse (*23*) in 1925, but no cultures were made. The statement that the plants recovered with the onset of warmer weather makes its identity doubtful.

ECONOMIC IMPORTANCE

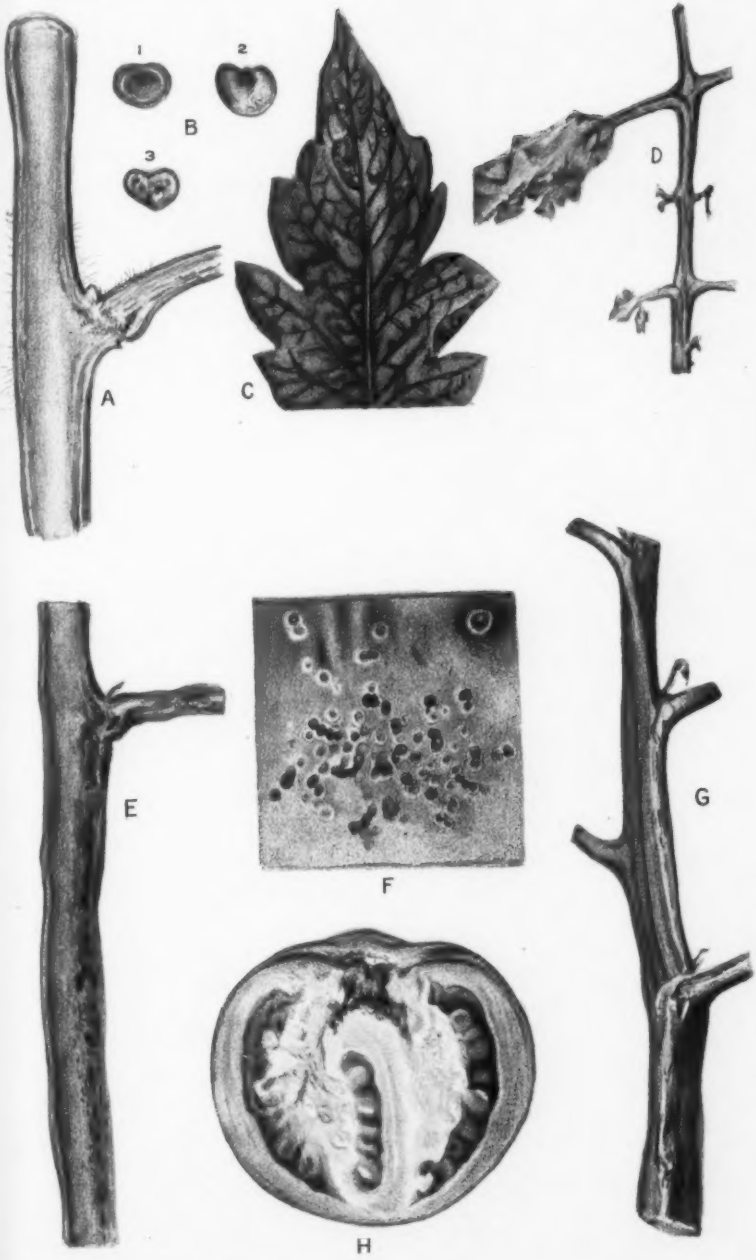
The widespread occurrence of bacterial canker and the fact that it is seed borne and is able to overwinter in the soil under some conditions, together with its very destructive nature, make this one of the most serious tomato diseases known. Field losses range from a trace to 100 per cent in individual fields. In many cases at the time when the crop should be in bearing there are very few living plants left in a field. (Fig. 1.) Such destructive outbreaks have occurred in Michigan, New York, Georgia, and Utah in large commercial plantings, and in smaller acreages in many other places. Fruit spot has caused losses of 25 to 75 per cent of the otherwise marketable crop in some fields in Georgia and Mississippi.

HOSTS

In the field the disease has been found only on tomato, although Smith (*25*) reported its spread to a spiny Porto Rican weed, *Solanum mammosum*, in the greenhouse. Repeated inoculations have failed to produce any infection on potatoes, eggplants, or peppers of either sweet or Cayenne varieties, or on the common solanaceous weeds *Physalis* sp. and *Datura stramonium*. On the other hand, no variety of tomato tested has been found resistant. The varieties tested include Marglobe, Marvelosa, Norton, Earliana, John Baer, Greater Baltimore, Bonny Best, Chalks Jewel, Stone, Globe, Red Pear, Walter Richards, Gulf State Market, Crystal Springs Market,

EXPLANATORY LEGEND FOR PLATE 1

A.—Longitudinal section of an infected stem. Early stage showing the yellow or cream-colored infected areas. Note the extensive invasion of the base of the petiole.
B.—Cross sections of bases of petioles: 1, normal; 2, early infected; 3, late stage of infection, with cavities.
C.—Leaflet showing leaf spots in several stages.
D.—Portion of leaf showing a streak on the rachis running out on a petiolule. Leaflets on the opposite side were healthy.
E.—Section of an infected stem at a later stage. Extensive invasion of the pith with cavities and darker color.
F.—Bird's-eye spots on green fruit.
G.—Streaks on a stem, running out onto petioles and beginning to crack open.
H.—Systemically infected fruit. Bacteria have flooded out from the vascular strands, making them appear broader than when freshly cut.



L. C. C. Krieger and J. F. Brewer, Fec.

(For explanatory legend see opposite page)

Ignotum, and a variety of the potato-leaf type. Kotte (*16*) reports Tuckswood, Triumph, and Lucullus as susceptible varieties.

PHASES OF THE DISEASE

Bacterial canker has two serious phases. The primary phase is a systemic infection which causes the gradual wilting and death of the plant and infection of the seed through the vascular system; and the secondary phase is a fruit spot which, although superficial, renders unsightly a large percentage of otherwise marketable fruit.

SYSTEMIC INFECTION

Effect on the Plant

The plant may go down at any stage, from seedling to the mature plant with a set of fruit. The disease usually progresses rapidly, but

FIGURE 1.—Tomato field in Georgia; vacant places caused by bacterial canker

several cases of recovery from early symptoms have been observed. Plants in the field may die before branching or setting fruit. Plants that have attained branching size show decided stunting of one or more branches, often with a thickening of the shortened internodes. (Fig. 2.) Light-colored streaks, sometimes becoming dark, appear on the stems, extending along one or more internodes or up the lower side of petioles or peduncles. (Pl. 1, G; figs. 3 and 4.) Narrower streaks also appear on the upper side of the rachis (pl. 1, D), and on the midribs and veins of the leaflets. These streaks, which follow the line of heavy infection in the cortex, crack open at intervals, forming cankers. (Figs. 3, 5, and 6.) The cankers on midribs and veins, though inconspicuous, are very common and from field evidence appear to cause more secondary infections than do the larger cankers on the stems.

One of the earliest symptoms, more noticeable on staked than on unstaked plants, is a turning downward of the lower leaves, accompanied by curling of the leaflets. An irregular wilting and

shriveling of the leaflets follows. When, as is very common, the infection runs on one side only of the petiole and midrib, a distortion of the leaf results, the leaflets on one side remaining healthy, while those on the other side die. (Fig. 3.) The petioles do not wilt while the leaflets are wilting and shriveling, but they may turn downward. The topmost leaves remain turgid until infection reaches

FIGURE 2.—Tomato plant diseased with bacterial canker, showing stunting and thickening of stem and irregular wilting

them, although in hot, dry weather wilting of the top has been observed; and at times, in the case of very severe infection, a general wilting of the plant occurs when only the lowest leaves show other symptoms.

In Mississippi, following extremely rapid, succulent growth due to abundant rain after drought, the first symptom on the leaflets was the appearance of sharply outlined pale-green spots of collapsed tissue lying between veins, often following the midrib. These areas, which sometimes were more than 1 centimeter wide by several centimeters long, later became gray as the leaflets wilted.

During the very wet weather occurring in Georgia in May, 1928, unusual symptoms were observed in many of the very succulent plants that were succumbing to the disease. Externally the younger parts of infected stems had a clear, water-soaked appearance; and

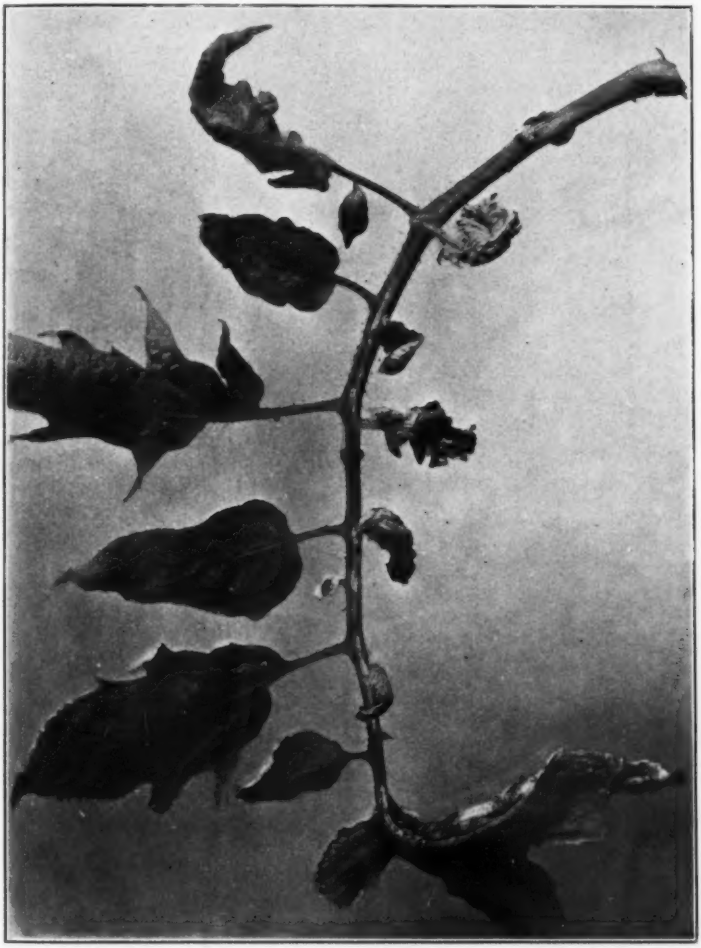


FIGURE 3.—Leaf showing infection on one side only. Streaks and cankers on the rachis and the midrib of the terminal leaflet

these, as well as adjacent infected petioles, were water-soaked inside instead of being mealy as infected tissues usually are. They showed the typical yellow color. This was the only time that such a condition was observed.

When a diseased stem is cut lengthwise the path of the disease is found to be very clearly marked by a line of creamy white, yellow,

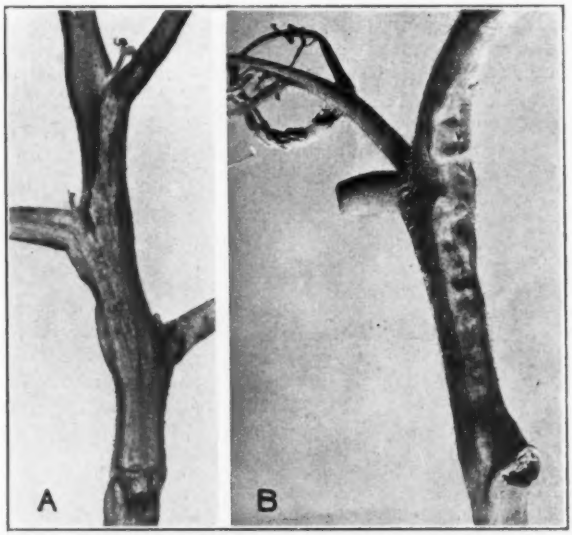


FIGURE 4.—Streaks on stems and petioles. Note the bulging of the stem in A and irregular surface in B over badly disintegrated tissues

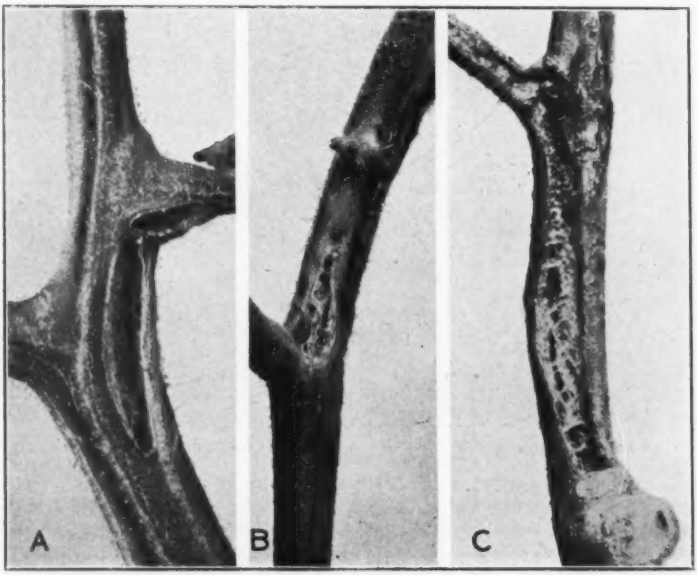


FIGURE 5.—Bacterial cankers on tomatoes: A, Canker on rachis, running out on a petiolule; B and C, cankers on stems

or reddish brown, just inside the woody cylinder (pl. 1, A and E), the reddish-brown color appearing in advanced stages. Along this line the pith cylinder is easily separated from the xylem. The discoloration is most conspicuous in the upper part of the plant where the bacteria have spread from the inner vessels of the wood to the more succulent phloem, pith, and cortex. Cavities of greater or less extent are formed in these areas. (Fig. 7.) The pith is often completely disorganized. The base of the petiole is especially susceptible and a cross section taken at this point is the easiest method of diagnosing the disease. Creamy white to yellow, mealy-looking areas in the pith or cortex, sometimes with cavities and in late stages with the red-brown tinge (pl. 1, A, B, and E), are clear evidence of bacterial canker.

FIGURE 6.—Old cankered stem and a stunted fruit on a cankered peduncle

Sometimes the root, especially the pith, is completely invaded; more often it shows very slight symptoms easily overlooked by the naked eye, but visible under a hand lens even when infection has taken place from infected soil through the roots; or it may be entirely free from infection. A slightly discolored line in the pith adjacent to the xylem is often the only sign of infection.

EFFECT ON THE FRUIT

Infection passes from the stem into the fruit through the vascular system and may be traced by the yellow infected veins directly to the seeds. (Pl. 1, H.) The infection spreads also into the tissues of the placenta and the fleshy covering of the seeds. In the hothouse overripe, heavily infected fruit has occasionally been observed of which the whole interior was disorganized and liquefied while the skin remained intact. Poured plates made from material removed aseptically from the interior of such a fruit gave pure cultures of *Aplanobacter michiganense*. Fruits infected when very young are

stunted and distorted. (Fig. 8, A.) Fruit that develops to nearly or quite normal size shows no external sign of internal infection. Yellow color is observable, however, in the scar made by removing the calyx. Heavy infection of the pedicel just beneath the calyx causes browning and shriveling of the sepals and loosens the attachment of the fruit. The tissues at the junction of pedicel and peduncle are very susceptible and frequently become so disintegrated that the fruit falls when the vine is shaken. Open cankers are common at this joint. (Fig. 8, B.)

In fruits of normal size the seeds do not show external evidence of internal infection, even when there is very heavy infection in the placenta and when the bacteria may be traced by the yellow veins directly to the seeds. Only in a stunted, distorted fruit, infected

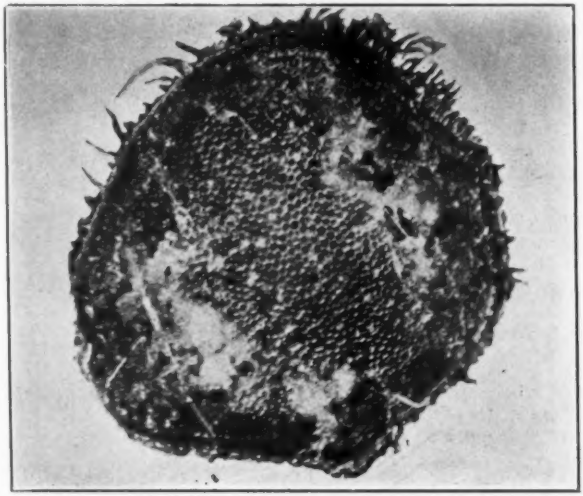


FIGURE 7.—Cross section of a diseased stem showing infected areas (white) and cavities forming in pith, xylem, and cortex. × 5

when very young, are the seeds abnormal. Then they are small, often black, and do not mature. A few such blackened or black-spotted seeds may be found in the larger fruits. Normal-looking seeds and discolored but normal-sized seeds from infected fruit give a high percentage of germination.

FRUIT SPOT

FIELD OBSERVATIONS

Fruit spot of the tomato was first observed by the writer in Georgia in 1928. Previous field studies had been made during the very dry season of 1927. In 1927 systemic infection was very severe in Georgia, but no fruit spot was noted. In the same location the following year systemic infection was again very serious. This was an unusually rainy season, and the idea that drought was a factor in the severity of the disease had to be abandoned, since the systemic infection was as severe as in the preceding dry year. On the first set

of fruit small, round white spots, some with brown centers, were noticed. Specimens were brought to Washington and isolations made. *Aplanobacter michiganense* was obtained in every case. Later the fruit spotting was so widespread in these fields that a large percentage of otherwise salable fruit had to be discarded. The next year (1929) there was a much smaller percentage of both the systemic disease and the fruit spot in these fields.

In 1929 bacterial canker was observed for the first time in Mississippi. (Fig. 9, A.) Fruit spot was more conspicuous than the systemic infection in these fields; that is, there were very few vacant places, but 75 per cent of the fruit that had set was spotted. There had been unusually heavy wind and rain storms, and the large number of

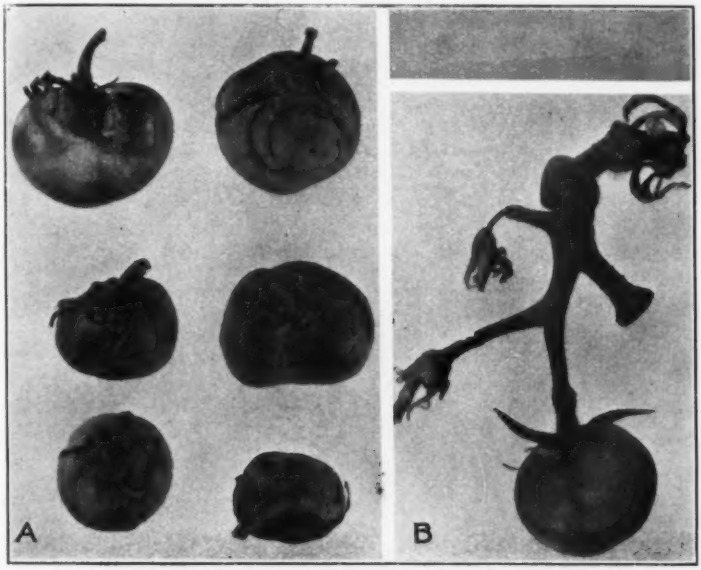


FIGURE 8.—Systemically infected fruits. A, Stunted, distorted fruit; B, infected fruit spur. Note cankers at junction of pedicels and peduncle, which cause the fruit to fall readily

fruits that were spotted on the side toward the prevailing wind gave good circumstantial evidence of spread by wind-blown rain. Open cankers on leaves or petioles could usually be found on the plants in the next row. This was especially significant when there were no cankers on the plant bearing the spotted fruit.

Good evidence that infection was carried from the leaf cankers to the fruit in the drip from rain or dew was obtained in Maryland and New Jersey, where only a small amount of disease was found and where the more conspicuous fruit spots led to the finding of the small leaf cankers just above. Fruit spot has also been traced to infection coming from open cankers on sepals or at the junction of fruit and calyx. Here where moisture is held by the calyx, providing ideal conditions for infection, elongated or irregular brown spots with white margins form along the edge of the scar. In some cases white

areas several millimeters in extent were observed in which a central breakdown had not begun. Groups of typical bird's-eye spots on the

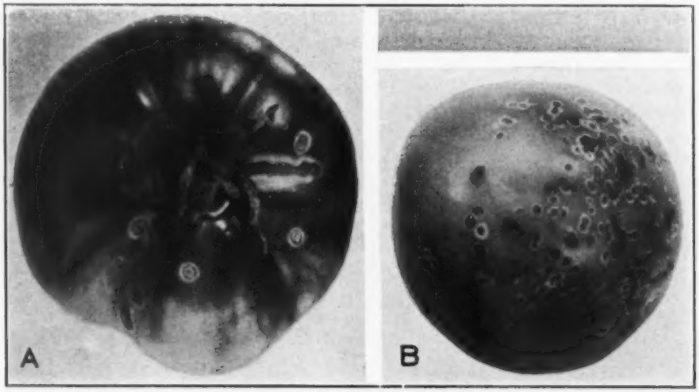


FIGURE 9.—A, Infected sepals, canker with white border running down from point of attachment, and large bird's-eye spots; B, numerous small bird's-eye spots

side of the fruit appear to have resulted from infection borne by drops of moisture from the open cankers under the calyx. (Fig. 9, B.)

FIGURE 10.—Bird's-eye spots on a young green fruit, produced by spraying with *Aplanobacter michiganense*, photographed 16 days after inoculation. ×2

The lesions on the fruit are at first minute, round, snowy white, slightly raised spots occurring singly or in large or small groups. (Fig. 11, A.) As these develop their centers become pustular and

break open with a single rift or with many small cracks, showing a roughened yellow-to-brown surface. (Fig. 10, Fig. 11, B, and Pl. 1, F.) The white persists indefinitely as a conspicuous halo, although in some old spots it disappears partially or completely. Spots may be so numerous as to form large, crusty patches. There is no water-soaking at any stage of development. The spots remain small, not exceeding 3 mm. in width, exclusive of the halo.

Isolations and Inoculations

Bacteria were repeatedly isolated from the spots with white halos. Isolations are most easily obtained from fruit spot by swabbing the surface repeatedly with sterile water, removing a group of spots with a sterile knife, and crushing them in a tube of beef broth from which the plates are then poured. Slant agar transfers from the resultant colonies that appeared typical for *Aplanobacter michiganense* were used to make prick inoculations on stems of healthy tomato plants in the hothouse. Typical systemic infection was obtained, the organism exhibiting as much virulence as freshly isolated, authentic isolations of *A. michiganense*. With the same culture fruit spot was produced by lightly swabbing young fruits from one-half to 1 inch in diameter with water suspensions from agar slants, using a small wad of cotton. Later, fruit infection was obtained by spray inoculations with the same isolations. (Figs. 10 and 11.) Reciprocally, fruit spot was readily produced by inoculation with *A. michiganense* isolated from systemically infected stems. White spots one-half millimeter wide were usually noticed from four to six days after inoculation by either swab or spray. The size of the subsequent spot and the rapidity of the development of the roughened center depend on the age of the fruit. The largest spots developed on fruits that were very small (one-half to 1 inch in diameter) when inoculated.

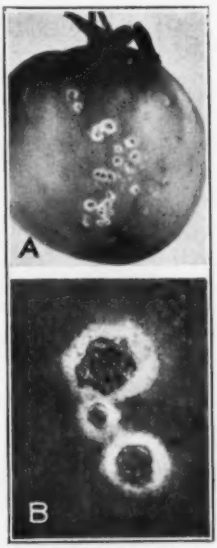


FIGURE 11.—Spots produced by spray inoculation. A, Very young spots five days after inoculation. × 5. B, Enlargement of portion of Figure 10 to show the rough, cankered centers of the spots. × 6

Comparative inoculations were made on young fruits with a culture of *Bacterium vesicatorium* received from Gardner. The resultant spots were always like those described by him. Water-soaking was always present, and no spots with white halos appeared.

Histology

Gardner and Kendrick (*14*) stated that since there are no stomata on tomato fruits and typical infection with *Bacterium vesicatorium* was not obtained by means of insect punctures, bacteria probably penetrate the epidermis through minute rifts in the cuticle or through broken hairs. In this they are doubtless correct. The young fruit is densely covered with two types of very delicate hairs, one a long slender trichome, the other a short hair with a several-celled glan-

dular head. Sections of fruit with young bacterial canker spots have shown the bacteria in the bases of hairs (fig. 12, A) and in the intercellular spaces of the adjoining subepidermal layers.

Fruit spots remain very superficial, not penetrating as deeply as those caused by *Bacterium vesicatorium*, but otherwise much like

FIGURE 12.—Sections of spots on green fruits. A, Bacteria in the base of an epidermal hair and in the subepidermal tissues. × 40. B, Section through a young spot. × 65

them in development. (Fig. 12, B.) The cells below the infected part multiply noticeably in an attempt to cut it off with a cork layer. There is also a multiplication of subepidermal cells in the region of the white halo, but the white appears to be partly due to the presence of air in the tissues, since it becomes inconspicuous when the specimen is put into alcohol.

Earlier Observations on Bird's-Eye Spot[4]

In 1918 Coons and Nelson (*10*) published a brief note on a tomato fruit spot which they called canker, found in several widely separated regions. They described the spots as white, and the single illustration tallies with the fruit spot caused by *Aplanobacter michiganense*. Photographs and notes kindly furnished the writer by Nelson show that this was the predominant type of spot. Coons (*9*) in a later paper says: "The disease is disfiguring enough to cause serious loss to the crop." His illustration in this case, however, looks like the spot caused by *Bacterium vesicatorium*. Coons and Nelson isolated yellow organisms from both types of spot, but inoculations were made only with the one from dark spots. Nelson's photographs of inoculated fruit give clear evidence that they were dealing with *Bact. vesicatorium*. Their cultures were lost before Gardner and Kendrick studied *Bact. vesicatorium*, so a comparison of the organisms could not be made.

Gardner and Kendrick (*12, 13, 14*) also found the white spots, which they called "bird's-eye spot." Their illustrations of this type of spot are absolutely typical of bacterial canker. The legend accompanying the illustration states (*14*): "Bird's-eye spot of tomato. Cause not proved. Possibly a form of bacterial spot." They did not succeed in isolating an organism from these bird's-eye spots, nor did they produce them by inoculation with *Bacterium vesicatorium*. They considered that they were working with the disease described by Coons and Nelson, as they say (*13*): "The disease as it occurs on tomato fruit has been known for several years. Coons and Nelson, and later Coons, briefly describe this disease as tomato canker." It is interesting that the spot originally called canker proves to be a phase of the disease now bearing that name.

McCubbin (*17*) in 1918 figures early and late stages of what he calls black scab and which Gardner and Kendrick (*13*) say appears to be bacterial spot. The figures of the early stage, however, show only white spots, which are typical of bacterial canker spots in the early stage. It would seem, therefore, that McCubbin had both diseases under observation.

SECONDARY INFECTION

Where fruit spot is present in the field it is usually accompanied by spotting of peduncles, pedicels, calyx, and young stems. This takes the form of small, tan-colored, open cankers from 1 to 2 mm. wide. These remain very superficial, and although they occasionally have been found to penetrate deeper and reach the vascular system on inoculated plants, progress is so slow as to be of no practical importance. Leaf spot has not been certainly identified in the field, because of the abundance of fungus spots that confuse the picture. The only place in which leaf spot has been observed to occur naturally and has proved to be caused by *Aplanobacter michiganense* was an abandoned "spotting" bed in Georgia, where the overgrown plants, many with open cankers, were in a tangled mass. (Fig. 13.)

Spotting on stems and leaves has been obtained in the greenhouse by spray inoculations. (Fig. 14.) Infection appears to start fre-

[4]The name bird's-eye spot, used by Gardner and Kendrick for this spot, the cause of which they did not determine, should be retained because of its descriptive value.

quently in the large bulbous bases of the giant hairs. Stomatal infection was recorded and figured by Smith (*26*). Leaf spots are minute and extremely inconspicuous, creamy white, and raised by the mass of bacteria in the tissues. These may remain indefinitely in this condition or dark rings of dead tissue may form around individual spots (fig. 14, A and B, and pl. 1, C) and merge into larger dead areas (fig. 14, C). Occasionally infection reaches the vascular system from such spots, especially from those lying over the very large bundle of vessels on the extreme leaf margin. Where such spread occurs the leaf blade yellows from the margin inward and the veins blacken. (Fig. 14, D.)

SPREAD IN THE FIELD

No positive evidence of field spread of systemic infection by any agency has been obtained. The greenhouse spread recorded by

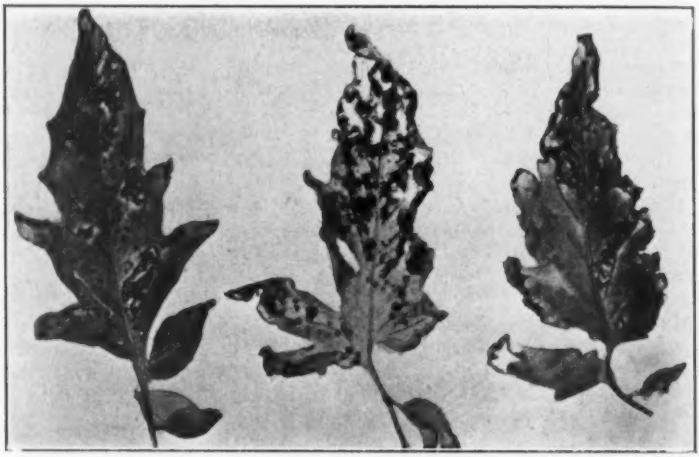


FIGURE 13.—Naturally infected leaves from an old spotting bed in Georgia. *Aplanobacter michiganense* was isolated from spots on these leaves

Smith has never recurred in the writer's experience. Moreover, the plants throughout the field in many cases succumb at approximately the same time. Slight variations are readily accounted for by the varying times at which infection takes place during the weeks in the seed bed. Spread in the greenhouse by the pruning knife has been reported by Coons (*9*) and by Williams (*28*). The disease has not been observed in fields where pruning was practiced, except in Mississippi; and there no convincing cases of spread were observed. Pruning in the field is often done by twisting out the side shoots. This, while not carrying the disease from plant to plant on the knife, leaves wounds for the possible entry of the bacteria. However, the disease progresses downward in the plant very slowly. The bacteria have been traced down from leaf inoculations through the petiole into the stem, but the spread from there is upward. When inoculation is made directly into the stem the spread downward is slow. Kotte (*16*) has recorded the same observation.

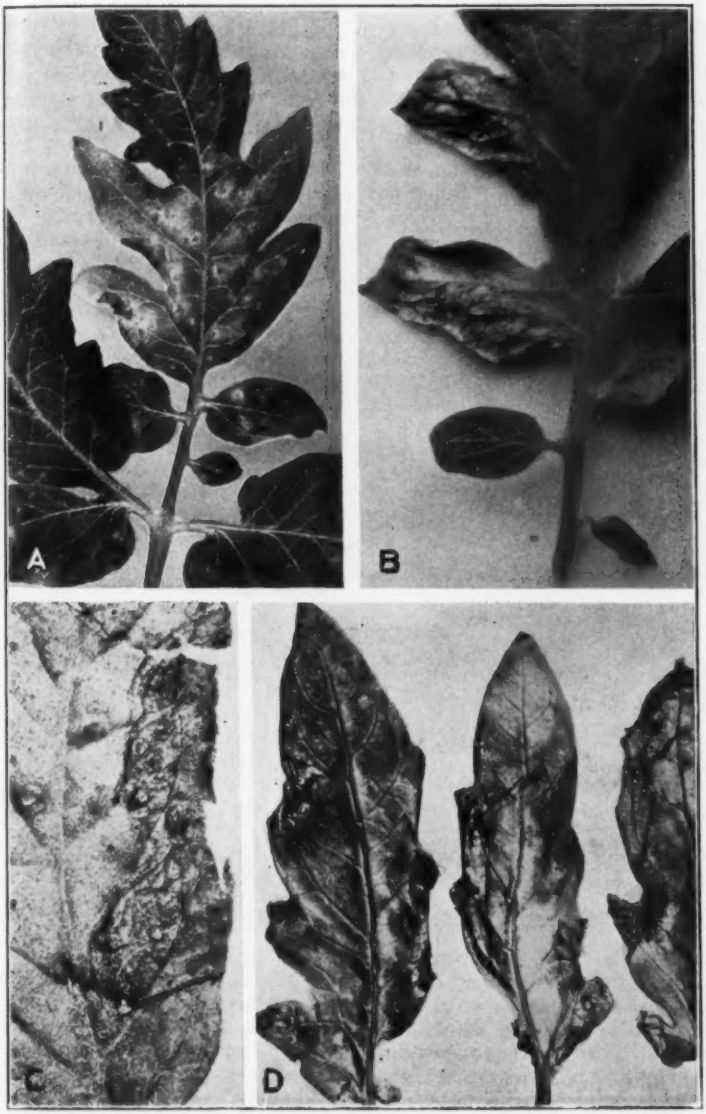


FIGURE 14.—Leaves inoculated by spraying with *Aplanobacter michiganense*. A, Upper surface of leaflet five days after spraying. Natural size. B, Leaflet dying from systemic infection; note blackened veins. × 2. C, Enlargement of a dead area in which small individual spots are distinguishable. × 2. D, Leaflets dying back from marginal infections. Natural size

INTERNAL INFECTION OF SEED

The presence of the bacteria inside the seed coat has been demonstrated by microtome sections and by cultural methods.

Sections of infected seed show that the bacteria lie in the fleshy outer seed coat (fig. 15), which is removed in the seed-cleaning process, and between the layers of the hard seed coat, penetrating the entire circumference of the seed in cases of severe infection. (Figs. 16, 17, and 18.)

Plates were poured from seed taken from fruits that showed infection in the placenta. Since not all seeds are internally infected, 10 or more were used for a culture. The seeds, after the removal of the fleshy, outer seed coat, were soaked in mercuric chloride, 1 to 1,000, for five minutes, rinsed thoroughly in sterile water, and crushed in beef broth. Plates were then poured and *Aplanobacter michiganense* obtained in pure culture. Isolations, identified by inoculations on tomato, have been made from seed 2½ years old.

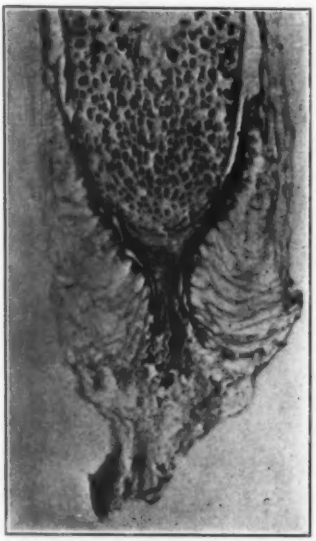


FIGURE 15.—Section of seed from which the fleshy covering had not been removed, cut through the hilum at right angles to the flattened surface and parallel to the long axis. Bacteria are abundant in the dark areas of the seed coat beneath the hilum and running up both sides of the seed. The fleshy, outer seed coat is heavily infected. × about 40

MEANS OF DISSEMINATION

Infected seed, and plants grown from infected seed or in infected soil and shipped to other localities, are the means of spreading bacterial canker from place to place. There is also evidence of its having been carried in manure. Seeds are infected both externally and internally. The heavy infection in the placenta of diseased fruits insures wholesale contact of bacteria with the seeds in the process of seed saving. The bacteria are able to survive in a virulent condition eight months or more on the seed or on cover glasses and two and one-half years in dry soil.

EVIDENCE OF DISEASE FROM INFECTED SEED

That disease results from planting infected seed is attested by wide field observations and by experimental planting of seed from infected fruit. From 1 to 5 per cent of infection has resulted in the greenhouse from naturally infected seed and from 21 to 40 per cent from seed dipped in bacterial suspensions, dried, and planted. Field tests in Georgia with seed saved from infected fruit gave 53.4 per cent of cankered plants in 1928 and 30 per cent in 1929;[5] checks grown from seed known to be clean gave only healthy plants.

[5] Communication by letter from O. C. Boyd.

A wide discrepancy has been observed in the percentage of diseased plants obtained from seed known to be infected but grown at different times and in different places. For example, seed known to be infected, which gave only 1 per cent of infected plants in the greenhouse in Washington, gave 54.4 per cent in Georgia. This indicates that seed-bed conditions are an important factor in the production of devastating outbreaks of bacterial canker. Since tomatoes are not grown to maturity commercially in the tomato-plant growing region of Georgia and reliable plant growers rotate their fields or use newly cleared pinelands, soil infection does not appear to be a factor in producing diseased plants in this region. To determine this point clean seed was planted in 1930 in two fields in Georgia from which infected plants were shipped in 1929. No bacterial canker developed on the resulting plants, part of which were grown to maturity in the seed field and part of which (400 plants) were set out in Maryland.

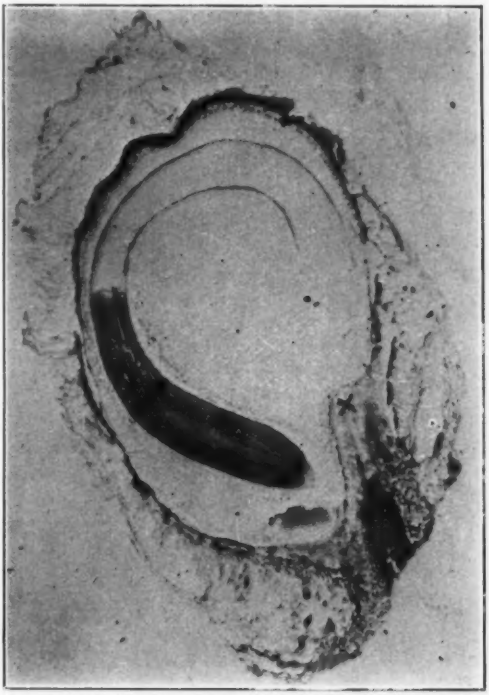


FIGURE 16.—Section of a seed parallel to the flattened sides, showing bacteria (dark areas) in the tissues beneath the hilum and completely encircling the seed. × about 25

SPREAD IN THE SEED BED

A few primary infections under crowded seed-bed conditions are potentially able to increase the number of diseased plants to 100 per cent, if conditions are favorable. Such spread was found in an old, abandoned plant bed in Georgia during a very rainy season. The plants, which were beginning to set fruit, were in a tangled mass, so that spread was facilitated. Spots were abundant on fruit, stem, and leaf. In many of the plants examined no internal lesions were found in the root or lower stem, although in the tender upper stem they were conspicuous, indicating that systemic infection had resulted through primary lesions on upper stems or leaves. Plants are set in the field or shipped, however, while quite young, and spotting has not been found at this stage. Moreover, severe outbreaks in the field have occurred from hothouse-grown plants where weather conditions do not enter as a factor. In several cases all diseased plants could be traced to one hothouse or coldframe. It would seem that

there must be some other cause of spread, possibly insects, in the seed beds. A study of this problem is under way.

SEED TREATMENT

For externally contaminated seed, mercuric-chloride treatment has been found most effective. Such treatment, however, does not control internal seed infection, which is the more serious phase of the disease. For the internal type of infection hot-water treatments were undertaken. A study was first made of the thermal death point of the causal organism at various ages.

THERMAL DEATH POINT OF THE CAUSAL ORGANISM

The thermal death point of *Aplanobacter michiganense* has been found to vary in different strains from 50° to 53° C. This, however, refers to young 24 to 48 hour old cultures in thin suspensions in beef broth. To ascertain whether it would hold for older cultures or for bacteria massed on the seed and dried there, the following tests were made:

FIGURE 17.—Enlargement of a portion of Figure 16 to show the position of the bacteria: *a*, Caulicle; *b*, endosperm; *c*, inner wall of seed coat; *d*, layer of bacteria; *e*, outer roughened wall of the seed coat. × 170

1. Seeds were sterilized in the oven to eliminate saprophytes and thus facilitate the reisolation of *Aplanobacter michiganense*, which is a slow grower. These seeds were then immersed in a suspension from 10-day agar slants of *A. michiganense*, drained, and dried. They were then dropped into sterile water in test tubes and held in a water bath at 55° and 56° C. for 10 minutes and 20 minutes, after first being warmed for 5 minutes at 45°. The bacteria were cultured from these seeds in every case.

2. Bits of the surface agar of slant cultures 20 days old were dropped into beef broth and exposed for various time-temperature periods. Typical bacterial growth in transfers made from these cultures after exposure demonstrated that the bacteria in this condition are not killed by exposures of 20, 30, or 40 minutes at 54° C. or by an exposure of 10 minutes at 55°.

HOT-WATER TREATMENT OF SEED

Two sets of hot-water treatments of seed were carried on, the first to determine the effect on the seed, the second to ascertain the point at which the bacteria are killed.

1. Freshly cleaned, healthy seeds were tested to determine the point at which injury occurs. The seeds, covered with water in test

tubes, were first exposed in a water bath at 45° C. for 5 minutes, then plunged into water at 50°, 52°, and 54° and kept at these temperatures for 10, 20, 30, 40, 50, and 60 minutes. Germinations were counted in 100 seeds of each lot placed between damp blotters. One hundred seeds of each lot and 100 check seeds were planted in the greenhouse and watched for sturdiness. Although there was slight retardation after the longer time treatments at 54° (3 days at 54° for 40 to 60 minutes), there was no reduction in percentage of germination or vigor of plants in any case. The 50° treatments stimulated earlier germination than took place in the checks. Plants were potted and held until they had reached a height of 8 inches. The same results were obtained with dry seed treated at these temperatures. Similar experiments at 55° and 56°, for 30 minutes and over, produced decided injury to wet, freshly cleaned seeds, both in germination and vigor of plants. Dry seeds were not noticeably injured when treated for 30 minutes at these temperatures but when exposed for longer periods gave a decidedly lower percentage of germination and weaker plants.

FIGURE 18.—Section of a seed cut in the same plane as Figure 16 and at a point corresponding to X in Figure 16, showing bacteria massed in the vascular region and more scattered in the adjacent loose tissue. × 210

2. Seeds from infected fruit were treated with hot water for the various time-temperature periods used in experiments with clean seeds. After such treatment mercuric chloride, 1 to 1,000, was used for 2 minutes to eliminate any saprophytes that might be present on the surface. The seeds were then rinsed in sterile water and crushed fine in beef broth. Since not all seeds are internally infected, 10 or more seeds were used for isolation purposes. The bouillon with the crushed seeds was poured into test tubes containing potato cylinders. Untreated diseased seeds were cultured in the same way, and the resultant yellow bacterial growth tested for its identity. In this way *Aplanobacter michiganense* was cultured from the checks and from seeds treated at 53° and 54° C. for 30 minutes, but not from those treated for 40 minutes. Since 55° is injurious and since commercially it is difficult to hold the water bath at a given temperature without variations of more than a degree, this does not

allow a sufficient margin of safety for general use. Further investigation may show that the method, although impracticable for commercial seed treatment, might be used in cleaning up a seed stock. The only real remedy appears to be in growing clean seed. The only way to obtain clean seed is to select from fields in which there is no bacterial canker.

PERSISTENCE OF BACTERIA IN THE SOIL

Laboratory tests to determine conditions under which the organism can live in the soil have shown tolerance of a wide range of temperature, moisture, and pH values.

Tubes of soil were sterilized, then inoculated with water suspensions of *Aplanobacter michiganense* sufficient to moisten. Half were sealed with paraffin to retain the moisture, the other half were left unsealed and soon dried. Part were held out of doors protected from rain in Washington and part at Geneva, N. Y., from June 15 to April 1. At the end of this period the organism was recovered by cultural methods in virulent form from both wet and dry soil from both places. In another experiment the organism survived two and one-half years in dry sterilized soil exposed to outdoor temperatures in Washington, D. C.

Preliminary experiments have been made in unsterilized soil sealed to retain the moisture and kept at low temperatures in refrigerators. These experiments have demonstrated that the organism will survive five months at 0° F. in soils from different sources with a pH of 5.67, 6.67, and 7.9, respectively. The viability of the organism was determined by inoculation, as it had been found impracticable to isolate *Aplanobacter michiganense* from unsterilized soil. For this purpose the soil culture to be tested was shaken with water for five minutes, allowed to settle, and the suspension thus obtained was used for prick inoculations on young tomato plants. Inoculations were also made by dipping the roots of very young plants in the suspension.

Tomato plants readily become infected when planted in freshly inoculated soil or when inoculum is poured into the soil around them in pots in the greenhouse. However, when plants so infected were allowed to die in the pots, the old vines removed, and young plants set in three months later, no disease resulted. This agrees with the report of Williams (*28*), who recorded inability to hold the organism in greenhouse soil for even a few months. An attempt to determine persistence in the soil in the open at Washington, D. C., was made. Twenty-four 8-inch pots filled with sterilized soil were buried to their tops in the ground in October. Leaves and stems of infected plants were buried in the upper layers of soil in 14 of the pots. In May the soil in the pots was worked. A water suspension of *Aplanobacter michiganense* was poured into the soil of 6 pots that had not been inoculated in the fall, and the remaining 4 were held as checks. Young Stone tomato plants were set in all of the 24 pots. The plants were watched all summer, but no disease developed in the checks or in the pots inoculated in the fall with diseased plants, although all of those in the freshly inoculated soil succumbed early to the disease. Since this one negative result is not conclusive, the experiment is being repeated, this time without pots.

Negative evidence was obtained in western New York, where for two successive years the disease did not appear in a field that had been a total loss from bacterial canker the preceding year. Further observations and experiments are needed before there can be any certainty in regard to this region.

There is positive evidence, however, that the disease can and does overwinter in the soil in some localities. In Georgia the study of soil hold-over was complicated by the bad field practice of the growers who used infected vines in the compost that was placed under the soil of spotting beds. By this practice almost 100 per cent of the plants became infected. When in 1929 the practice was abandoned there was only from 5 to 10 per cent field infection. This, while indicating hold-over in the soil of fields, is not so large a percentage as might have been expected in fields that had developed 50 to 75 per cent of infected plants the two preceding years. That the bacteria survived composting and were virulent in this compost the second year was demonstrated by Boyd.

Indication of a small percentage of disease from hold-over in the soil was observed in 1929 in California and Utah but was not conclusive because the seeds used were not known to be clean. In Illinois only one diseased plant was found in a half-acre field which had had 25 per cent infected plants the preceding year. Further observations are needed to determine the length of time the infection will remain in the soil. The small percentage of infection so far observed in fields infected the preceding year and the fact that the disease does not attack other crops or the weeds so far tested would indicate that rotation of crops should eliminate the bacteria from the soil. This theory is further supported by the fact that no bacterial canker could be found in 1930 in two fields in Georgia planted in tomatoes for the first time since 1927 when the infection was severe.

CONTROL

Control of bacterial canker is a difficult problem. Seed infection, soil infection, and spread in the seed bed must all be taken into consideration. Where the disease has not occurred, the use of clean seed is the first essential for safety. Seeds brought into disease-free areas for breeding purposes should be grown in separate seed beds, the trial plots carefully inspected, and any infected plants burned.

Seed should not be saved from fields in which even a small percentage of bacterial canker is found. Seed not positively known to come from clean fields should be treated with mercuric chloride, normal Semesan solution, or Semesan Jr. dust. This will eliminate surface infection and is desirable from the point of view of other tomato diseases, but no treatment is known which will destroy internal infection, under commercial conditions, without danger of injury to the seed.

Where the disease has occurred, rotation of crops also is essential. The number of years that the bacteria will live in the soil under field conditions when rotation is practicable has not yet been demonstrated. The small percentage of diseased plants on fields infected the preceding year, the absence of the disease from two old fields after two years in other crops and the fact that other crops and solanaceous weeds are not attacked make this a promising method. Where ro-

tation is impracticable, the development of a resistant strain of tomato would be desirable. The removal and destruction of diseased vines to reduce the amount of infection in the soil would also be desirable under these conditions. Kotte (*16*) suggests that infection through roots broken in transplanting may be prevented by dipping the roots in a 0.5 per cent solution of Uspulun thickened with soil to the consistency of a thin mud. A preliminary experiment was made in the greenhouse with Kotte's method, in which normal Semesan solution was used instead of Uspulun when setting young plants in heavily inoculated soil. This method resulted in a 20 per cent infection in the treated plants as compared with an 80 per cent infection in the checks.

Care should be taken in the sanitation of the seed bed. Williams (*28*) reports satisfactory control of the disease in greenhouses by sanitation but gives no details. No infected refuse, either vines or spotted fruit, should be returned to the soil or allowed to reach the compost heap or the barnyard. The soil of old beds in cold frames or hothouses should be replaced with clean soil from fields where tomatoes have not been grown, or it should be sterilized by steam. Hothouses should be fumigated. In the South, where seeds are sown in the open, fields to be used as seed beds should be burned over before plowing to destroy insects in plant refuse. Fall plowing would also aid in decreasing the insect population. A field should not be used two successive years for plant growing for the best results from several points of view.

Very few plants show any sign of infection at the time of setting in the field. Inspection for disease at this stage would be impracticable.

THE ORGANISM

VARIANTS

An albino strain has been isolated from single-colony cultures of *Aplanobacter michiganense* (*8*). So far as known, such a variant has not been reported for any other plant pathogene. Brown (*3*) found rough, rubbery, round white colonies on reisolation plates of *Bacterium beticolum* with which she obtained infections on sugar beets, but she did not record their cultural identity with that organism, nor did they appear in single-colony cultures of the typical yellow organism. White variants that do not revert to the typical color have been described by various workers for bacteria in other groups, notably *Bact. prodigiosus*, *Bact. violaceum*, *Staphylococcus aureus*, and several saprophytes from plants.

White streaks were observed in the surface growth on old agar stab cultures at three different times and with isolations from different places. On one occasion plates were poured from the white part of a culture of a strain isolated four years previously, and both white and yellow colonies appeared. These were alike except in color. Transfers from white colonies were found to be nonmotile and Gram-positive. No further work was done at that time and the cultures were lost. When in 1929 the same phenomenon occurred in a strain held two years on culture media, white colonies were again isolated. These were tested as before and also used for inoculating tomatoes. Prick inoculations were made in the stem and swab inoculations on young green fruits. In both cases typical lesions were produced,

the organism proving as virulent as the typical yellow form. Reisolations were made from petioles at intervals of 12 days, 6 weeks, and 2 months after inoculation, from near the point of inoculation and from the upper limits of the bacterial invasion. In all cases only white colonies appeared on the plates. In cultural tests on potato, litmus milk, Thaxter's potato agar, gelatin, beef broth, nitrate broth, and starch agar, the white form resembled the yellow form except in color. No yellow pigment was produced at any time.

Variants of another type were observed on whey agar and Thaxter's potato agar. On both of these agars containing sugar, on which the typical colony growth is abundant, fluid, and spreading, varying percentages of small, round, very convex colonies have appeared. These remain small and never spread. When replated on these agars the round type gives only round colonies; the spreading type may throw off a few round colonies. Replated on beef agar they are indistinguishable. The round colony has appeared in small numbers in young isolations but is much more common in isolations that have been on culture media for some time. In one 10-year-old strain only the round type was present. In this case no infections could be obtained. Round colonies of younger isolations are very weakly parasitic and weak in their reactions on culture media as compared with the spreading strain. They have not, however, lost the power of fermenting sugars.

LOSS OF VIRULENCE

Smith (*25*) has reported that *Aplanobacter michiganense* loses its virulence in the course of several years. Tests with various isolations—three of 1924, three of 1925, and one of 1926—were used in 1929 to make prick inoculations in the upper stems of large plants. All except two of the 1924 isolations showed symptoms of infection by the tenth day, and the disease developed rather slowly. Inoculation of the stems of young plants with the same cultures produced rapid and complete destruction of the plants, with typical symptoms. Virulence is therefore not lost by five years on culture media. As noted under "Variants," a 10-year-old culture failed to give any infection.

MORPHOLOGY AND STAINING REACTION

Aplanobacter michiganense is a small, nonmotile rod, measuring 0.6 to 0.7 μ by 0.7 to 1.2 μ, and occurring singly or in pairs. It is definitely Gram-positive. No spores have been observed. It stains readily with the usual bacterial stains. Capsules occur in agar cultures.

CULTURAL CHARACTERS [6]

BEEF AGAR.—Growth in beef agar is slow, moderate, opaque, smooth, glistening, pale yellow becoming mustard yellow,[7] and not opalescent. Colonies on beef-agar plates may be seen under the hand lens on the second day but usually are not visible to the naked eye until the third day. They are then very minute, 1 mm. or less in diameter, very pale yellow, and very convex. By the fifth day they have reached a diameter of 2 to 3 mm., are round with an entire margin, smooth, shining, of butyrous consistency, opaque, pale yellow, and show no markings by either reflected or transmitted light. In 12 days colonies on thinly sown plates may attain a diameter of 6 mm. At this time they are bright yellow

[6] Unless otherwise stated, all beef media were made with beef infusion and had a pH value of 7.0 to 7.2.
[7] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C.

and very viscid. Growth on the surface of agar stabs is round and restricted, attaining a width of 7 or 8 mm. by the eighth day. The stab growth is saccate and granular, visible to one-third the depth of the agar. On slant agar there is moderate growth, not running down into the V. On beef-extract agar growth is less abundant, paler (straw yellow) and thinner than on beef-infusion agar; it is opalescent and does not become viscid.

THAXTER'S POTATO AGAR.—On this medium growth is very abundant, fluid, long continued, and paler than on beef-infusion agar, though not so pale as on beef-extract agar. By the sixteenth day colonies may attain a diameter of 15 mm. and show no sign of drying down but remain thick and fluid looking, revealing a flocculent interior by transmitted light. Bottom colonies are thin, opalescent, and about 2 mm. in diameter. In slants the growth flows down, filling the V to a depth of 1 cm. or more, and leaving only a thin layer of growth on the slant. The mass of growth in the V is as deep in color as growth on beef-infusion agar. Large lenticular, granular bodies, looking like giant buried colonies, appear in the surface layer at the base of the slant in about two weeks.

BEEF BROTH.—Clouding is slow and moderate, beginning in about 48 hours. A pale-yellow rim begins to form in patches by the sixth day and is complete by the seventh or eighth day. Occasionally in undisturbed cultures a delicate incomplete pellicle is formed.

POTATO.—On potato cylinders growth is moderate, mustard yellow, smooth, glistening, and spreading, but not covering the entire surface of the potato. The water remains clear, with a moderate precipitate. The potato is grayed.

SYNTHETIC MEDIA.—Cohn's solution takes on a slightly milky appearance, but shows no rolling clouds on shaking. In Uschinsky's solution clouding is weak after 10 days' growth and only moderate, with a small amount of precipitate and with neither rim nor pellicle, at the end of two weeks. Moderate clouding occurs in Fermi's solution.

PHYSIOLOGY

LIQUEFACTION OF GELATIN.—Gelatin is very slowly liquefied at 18° to 21° C. In one test the surface growth after four days was about 4 mm. wide, lying in a dry depression 3 mm. deep. Two weeks later the gelatin was liquefied to a depth of 8 mm. and by the end of the fourth week the stratiform liquefaction was 12 mm. deep. The fluid was clear, with a heavy layer of yellow precipitate on top of the solid portion. No further liquefaction took place during the following month. In other tests evaporation kept pace with liquefaction so that only a dry pit was formed.

RELATION TO FREE OXYGEN.—The organism is aerobic. It does not grow in the lower end of stabs in beef agar or Thaxter's agar or in the lower half of shake agar cultures. No clouding occurs in the closed end of fermentation tubes.

FERMENTATION OF SUGARS.—Tests were made on beef-extract agar slants with bromcresol purple as indicator and 1 per cent, respectively, of the following carbohydrates: Sucrose, dextrose, galactose, levulose, maltose, lactose, glycerin, and mannit. The slants were streaked from agar cultures. In sucrose and levulose, acid was evident at the upper end of the slant after 24 hours. Two days later the whole of the slanted part of the culture was acid in sucrose, levulose, dextrose, and galactose. In maltose the slant was acid on the tenth day. On the fifteenth day the medium was acid throughout in all of these sugars except maltose, which never became as acid as the others. An alkaline reaction started on the seventeenth day and 10 days later the medium was alkaline throughout. In lactose, glycerin, and mannit there was a very slight color change, indicating an acid reaction, after two weeks' growth. Phenol red was then used as an indicator in the same kind of agar with each of these three carbohydrates. Acid was evident on the fourth day in glycerin and mannit, and on the tenth day the whole of the medium was acid. The lactose cultures began to show acid on the tenth day and were acid throughout on the seventeenth day. In fermentation tubes containing these carbohydrates in peptone water, the closed end does not cloud and no gas is formed.

REACTION IN MILK.—There is no evidence of growth in milk for several days. By the fifth day a yellow rim and surface layer of yellow growth 3 mm. deep has formed. These gradually increase to a depth of 1 cm. The milk remains fluid for about a month, although a soft curd may form during this period. In older cultures a solid curd forms with more or less separation of a clear yellow whey. Reduction of litmus is slow, beginning about the eighth day throughout the medium, and is complete in three weeks. No acid reaction appears before reduction has taken place, but the medium turns red when the cultures are 6 to 8 weeks

old. Reduction of methylene blue proceeds from the bottom upward, beginning on the third day, and is complete in 7 to 10 days. No crystals are formed.

HYDROLYSIS OF STARCH.—On starch-agar plates streaks which had made good growth were flooded with iodine when 7 days old. This produced a line 12 mm. wide around the growth, varying from deep purple in the outer portion to pale purple in the inner part nearer the growth, and in some cases colorless in the 1 mm. adjacent to the growth.

REDUCTION OF NITRATES.—Nitrates are not reduced. Tests were made in nitrate-bouillon cultures with the starch potassium iodide-sulphuric acid test and with the α-naphthylamine-sulphanilic acid test, and in the series of special media recommended in the Manual of Methods (*27*) for organisms giving a negative reaction in the simpler tests.

OPTIMUM pH FOR GROWTH.—In a series of broths made with beef infusion and Bacto-peptone, ranging from pH 4.4 to 9.6, growth was evident on the second day at pH 6.1 to 8.3 and was best at pH 7.7 to 8.3. By the fifth day there was clouding at pH 9.0 (at this time the checks had a pH value of 8.6), but there was no growth in the acid range beyond pH 6.1. By the seventh day a rim had formed in the culture at pH 7.5 to 8.2, but not in the others. Berridge (*1*) records growth with this organism at pH 5.0 to 9.2 in peptone broth made with Jardox. On agar slants growth was decidedly heavier at pH 7.6 and 7.9 than at pH 6.9. On agar plates 2-day-old colonies were barely visible at pH 7.0, whereas they were 1 mm. wide at pH 7.9.

TOLERATION OF SODIUM CHLORIDE.—The organism is sensitive to sodium chloride. Growth is moderate in beef broth at pH 7.0 containing 1 per cent Na Cl, weak in broth containing 2 per cent, and does not occur in broth containing 3 per cent.

OTHER PHYSIOLOGICAL REACTIONS.—*Aplanobacter michiganense* does not form indol or hydrogen sulphide. There is slight ammonia production in beef-peptone media, as shown on sugar agars containing bromcresol purple and by strips of filter paper dipped in Nessler's solution and inserted in beef-broth cultures.

TEMPERATURE RELATIONS.—The thermal death point ranges from 50° to 53° C. with different isolations. Tests were made with beef-infusion broth at pH 7.8, inoculated from 24-hour-old beef-broth cultures that had been heavily enough inoculated from an agar slant to cloud well in that time. Smith (*25*) reported no growth after exposure at 47°. The best growth is made at 25° to 27°. There is weak growth at 33° and moderate growth at 29°. No growth occurs in beef broth at 35° or 37°, but cultures were not killed by exposure for 10 days at these temperatures, as there was prompt clouding on removal to room temperature. Very slow clouding occurs at 1°. The organism has withstood year-round outdoor temperatures in Washington, D.C., and Geneva, N.Y.,[8] in sealed test tubes of sterilized soil, both moist and dry.

RESISTANCE TO DESICCATION.—*Aplanobacter michiganense* has survived eight months' drying on seeds and on cover glasses [9] and 2½ years in dry, sterilized soil without complete loss of virulence.

QUICK DIAGNOSIS

Slow-growing yellow colonies from typical lesions appearing on the third day on beef-agar plates, made up of small nonmotile, Gram-positive rods, may be safely diagnosed as *Aplanobacter michiganense*.

BRIEF CHARACTERIZATION

Aplanobacter michiganense is a short, nonmotile, Gram-positive, nonacid-fast rod occurring singly or in pairs, 0.6 to 0.7 μ wide by 0.7 to 1.2 μ long. It forms round, opaque, mustard-yellow colonies on beef agar; clouds beef broth slowly and forms a pale-yellow rim; has very weak diastatic action; liquefies gelatin very slowly; forms acid without gas from dextrose, sucrose, galactose, levulose, and maltose, and a very slight acid from lactose, glycerin, and mannit; does not reduce nitrates; causes very slow coagulation of milk without peptonization; reduces litmus and methylene blue in milk but does not form tyrosine crystals; produces ammonia but no indol or hydrogen sulphide; is aerobic; grows best at pH 7.5 to 8.2; withstands drying for 8 months on cover glasses and 2½ years in soil; optimum temperature is between 25° and 27° C.; thermal death point, 53°. Pathogenic to tomatoes.

[8] This result was obtained through the cooperation of J. G. Horsfall of the Geneva station.

[9] Tests on seed and cover glasses were not carried beyond 8 months, but the organism has been isolated from the interior of 2½-year-old seed.

SUMMARY

Bacterial canker has become more widespread and destructive in important tomato-growing sections in recent years. It causes the death of the plants at any stage from seedling to mature plant with a set of fruit. Losses range from a trace to 100 per cent. The disease is characterized by stunting of the plants; a gradual wilting and irregular dying of the leaves; streaking and cracking open of the stems, petioles, and veins; and the final collapse of the whole plant. The bacteria enter the fruit and penetrate the seeds through the vascular system.

During wet seasons bacterial canker also causes a very disfiguring fruit spot which renders the fruit unmarketable but does not cause it to rot. The spots are small but numerous and are characterized by noticeable white halos. Aside from this fruit spot there is no serious field spread.

The causal bacteria are disseminated on and in the seed. External disinfection should be practiced. Hot-water treatment has been found impracticable under commercial conditions, since temperatures that kill the bacteria are too close to the point at which injury to the seed occurs. According to present information, absolutely clean seed can be obtained only by selecting seed from fields free from this disease. Seed-bed sanitation is important, since the percentage of infection from infected seed is in part determined by seed-bed conditions that cause spread from plant to plant. These conditions are under investigation. Rotation of crops should be practiced, since it has been demonstrated that the bacteria are able to overwinter in the soil in some parts of the country.

The cultural and physiological characteristics of *Aplanobacter michiganense*, the causal organism, are given. Two variants, one an albino, are described.

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A COMPARATIVE STUDY OF THE STEM EPIDERMIS OF CERTAIN SUGARCANE VARIETIES [1]

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INTRODUCTION

A casual examination of the anatomical structure of the stem epidermis in certain varieties of sugarcane revealed, among even the most uniform specimens, differences that seemed to merit more detailed study. Such differences, if established, would, it was hoped, supply diagnostic characters of value in classifying varieties and in determining relationships. Representative varieties of four species of sugarcane (*Saccharum officinarum*, *S. sinense*, *S. barberi*, and *S. spontaneum*) and of several species hybrids which were examined seemed to offer a sufficiently wide range of material within the genus to justify a preliminary study for the purpose of establishing whether these structures might be useful for the purpose.

The existence of certain varietal differences was already known to Wieler,[2] who, in studying the structure of the sugarcane stem, examined the epidermis of several varieties and noted that in certain canes the siliceous epidermal cells were very abundant while in others they were much fewer in number. Wieler also described in detail the structure and development of these siliceous cells, but his conceptions were greatly modified in a later investigation by Frohnmeyer.[3] The observations of Wieler were extended by Mameli de Calvino [4] in her study of the anatomy of Cuban canes. She noticed that varieties differed in the size of the different types of epidermal cells, the thickness of the walls, and the distribution of the stomata.

MATERIALS AND METHODS

The material for the present investigation was obtained from the fields of the Sugar Plant Experiment Station at Canal Point, Fla. Heinemann,[5] in her study of the epidermis of corn, found great variation in the composition of the epidermis of the different internodes. In order to eliminate the effect of similar differences in sugarcane, special care was taken to select material that had reached the same stage of development. It was found that mature internodes which had reached their final length and were no longer covered by the

[1] Received for publication June 28, 1930; issued December, 1930.

[2] WIELER, A. BEITRÄGE ZUR ANATOMIE DES STOCKES VON SACCHARUM. Beitr. Wiss. Bot. B. 2: 141–164, illus. 1898.

[3] FROHNMEYER, M. DIE ENTSTEHUNG UND AUSBILDUNG DER KIESELZELLEN BEI DEN GRAMINEEN. 39 p., illus. Stuttgart. 1914. (Bibliotheca Botanica, Heft 86.)

[4] MAMELI DE CALVINO, E. ESTUDIOS ANATOMICOS Y FISIOLOGICOS SOBRE LA CAÑA DE AZUCAR EN CUBA. Mem. Soc. Cubana Hist. Nat. "Felipe Poey" 4: 156–211, illus. 1922.

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protecting leaf sheaths were best suited for a comparative study; and, since the middle portion of the internodes showed the greatest uniformity in the appearance and distribution of the various epidermal structures, material was always taken from this region. On the average 10 individual samples of each variety were tested, and it was noted that the variation in the different specimens of a single variety was but slight and did not exceed variations observed in different fields of a microscopic preparation. It should be understood, however, that the tests were based on one season's crop and that practically all of the material came from the same field. It is therefore possible that under changed environmental conditions a somewhat different epidermal picture might be obtained; but because of the conservative nature of these structures such variation is not likely.

The epidermis was removed by the aid of Schulze's maceration fluid, but instead of using the prepared reagent as recommended by Grob,[6] the writer adopted the following procedure, which gave most satisfactory results. A piece of epidermis with the adhering cortical and fibrous tissue was cut from the central part of an internode and placed in a test tube containing several crystals of potassium chlorate and a few cubic centimeters of concentrated nitric acid to which a few drops of water had been added. The mixture was carefully brought to a boil, and after a few seconds, as soon as the epidermis separated off in the form of a thin pellicle, the contents of the test tube were emptied into a Petri dish partly filled with water. The epidermis was then mounted on a slide and stained with chloroiodide of zinc. If the maceration process is interrupted at the right point, the epidermis stains a bright blue; but if the maceration is not successful, the stain will not take at all or the differentiation will not be satisfactory.

The number of short-cell groups in a square millimeter was determined by projecting the stained preparations on a screen of ample size. This was done in order to estimate their relative number as accurately as possible regardless of irregularities in distribution.

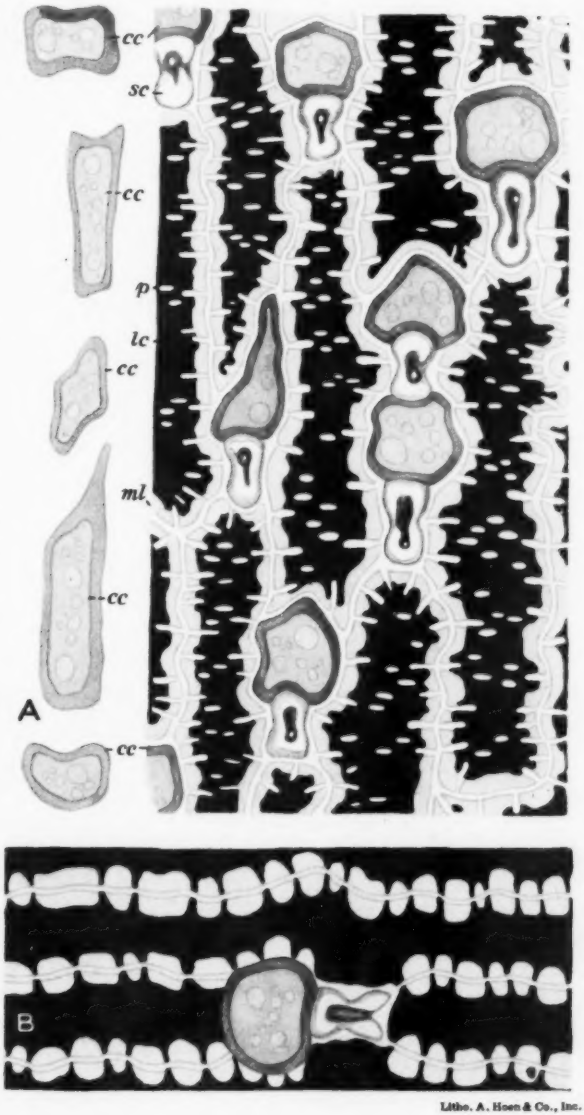
STRUCTURE OF THE EPIDERMIS

When the stem epidermis is removed in the manner described and is properly stained with chloroiodide of zinc, it appears under the microscope as made up of various kinds of cells, disposed, however, in a remarkably uniform pattern. Two distinct types of cells alternate with each other—elongated rectangular cells and short cells which occur singly or in pairs. (Pl. 1.)

The long cells, which constitute the greater part of the epidermal cells, form 4-sided prisms. They vary greatly in length, and their end walls, though commonly straight, are often pointed. The variation in width is less evident but becomes quite a factor when different varieties are compared with one another. The walls of the long cells have an undulated, strongly silicified middle lamella, which, in the stained preparation, appears as a pure-white wavy line. The thickening layers are also undulated but not uniformly at all points (fig. 1),

[6] GROB, A. BEITRÄGE ZUR ANATOMIE DER EPIDERMIS DER GRAMINEENBLÄTTER. 122 p., illus. Stuttgart 1896 (Bibliotheca Botanica, Heft 36.)

ENVIRONMENT



Litho. A. Hoen & Co., Inc.

A.—Surface view of epidermis of Louisiana Purple stained with chloroiodide of zinc. × 1,000. *cc*, Cork cell; *ml*, middle lamella of long cell; *p*, pit; *lc*, long cell; *sc*, silica cell

B.—Surface section of epidermis of Cayana. × 1,000. Notice the large number of pits in the walls of the long cells

and the degree of silicification is somewhat less. The outer wall of these cells is very thick, strongly silicified and suberized, and possesses a more or less strongly developed cuticle. The inner wall is much thinner than the outer one, stains a bright blue in the preparation, and is pierced by numerous pits. (Fig. 2.) Pits are also found in the lateral walls but are wanting in the outer one. Occasionally the lumen of the cells is filled with calcium oxalate in the form of crystal sand.

FIGURE 1.—Epidermis of Kassoer. × 1,000

The short cells typically occur in pairs, one member of which stains with chloroiodide of zinc a golden yellow and is known as a cork cell; the other member of the pair appears a glistening white and is known as a silica cell. Often, instead of two short cells constituting a group, a larger number is found. (Fig. 3.) This increase may be brought about by the omission of a long cell, so that two short-cell groups join each other directly. Sometimes there are one or two extra cork cells present, and thus the group may be greatly enlarged. While an increase in the number of short cells in a group is the more common occurrence, it may happen that by the omission of the silica cell the short-cell group is reduced to one member. This situation is common in some varieties and constitutes, as will be seen later, a valuable diagnostic character.

The cork cells have relatively thin walls, suberized and silicified, and a large lumen which usually contains a solid deposit of some organic substance. Though variously shaped, in certain varieties they may be remarkably uniform, being usually broadly reniform with the greater dimension parallel to the long axis of the stem. Frequently they are square or rectangular, especially in varieties in which the silica cells are few in number or wanting. They may also be trapezoidal, triangular, or much elongated (pl. 1, A) with end walls square or pointed like thick-walled

hairs (fig. 4). These elongated cells are placed by Mameli de Calvino[7] in a special class; they are, however, only an extreme type of cork cell. The walls usually stain with chloroiodide of zinc a golden yellow, while the lumen often contains silica. Sometimes the walls of these elongated cork cells are very thick and heavily infiltrated with silica so that they appear a glistening white in the preparation. The cork cell of a group in whatever form it occurs always lies vertically above the silica cell.

The silica cells are very uniform and usually rectangular with the long diameter parallel to the long axis of the stem. (Pl. 1, A, *sc.*) Occasionally they are broader than long, oval, or even round. In surface view they appear biscuitlike with slightly overhanging margins. The cells are practically solid, though in the center one commonly observes small air spaces often arranged in a row and containing some refractory material, the remains of the protoplast.

Comparatively few stomata are found in the epidermis of the stem, though there are varietal differences of which significant use can be made in classification. The structure of the stoma is normal except that its cells become strongly silicified, remaining pure white in the preparation.

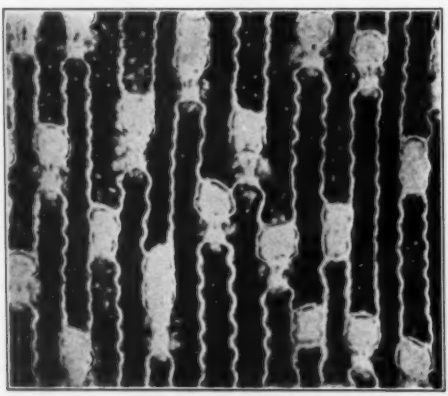


FIGURE 2.—Epidermis of Cayana. × 500

THE EPIDERMIS OF CERTAIN VARIETIES

D-74

The variety D–74 stands more or less by itself because of the small size of its epidermal cells. (Fig. 5.) There are between 90 and 130 rows of long cells in a square millimeter, so that the average width of the cells varies from 7.7 μ to 11.1 μ. The number of short-cell groups in a square millimeter reaches 1,112 or more. Because of the large number of short cells, the long cells also are relatively short. However, the variation in length is so great that the determination of a mean value would have little significance. The short cells in section appear square, rectangular, or trapezoidal, and occasionally pointed. Sometimes they occur in pairs with one member of the pair a cork cell, the other a silica cell; sometimes the cork cell stands alone. The number of stomata varies, but as many as 14 have been counted in a microscopic field covered by a 16-mm. objective and a No. × 6 ocular, or an area of 2.4 sq. mm.

LOUISIANA PURPLE

In the Louisiana Purple variety also there is a preponderance of short cells (fig. 3), for as many as 928 were counted in a square millimeter, and, since they are comparatively large, their total area equals that of the long cells and may even exceed it. This fact becomes

[7] Op. cit., p. 179. (See footnote 4.)

evident at once if one views the epidermis under low magnification. There are, on an average, 100 rows of long cells in a square millimeter, so that the average cell width is about 10 μ. The long cells are, on the whole, comparatively short and their end walls are usually transverse. The cork cells are reniform, square, or pointed, but hairlike cork cells are rare. The short cells appear most often in pairs or double pairs with one member a cork cell, the other a silica cell. The number of stomata varies with the microscopic field. At most, however, there are only one or two stomata in a field.

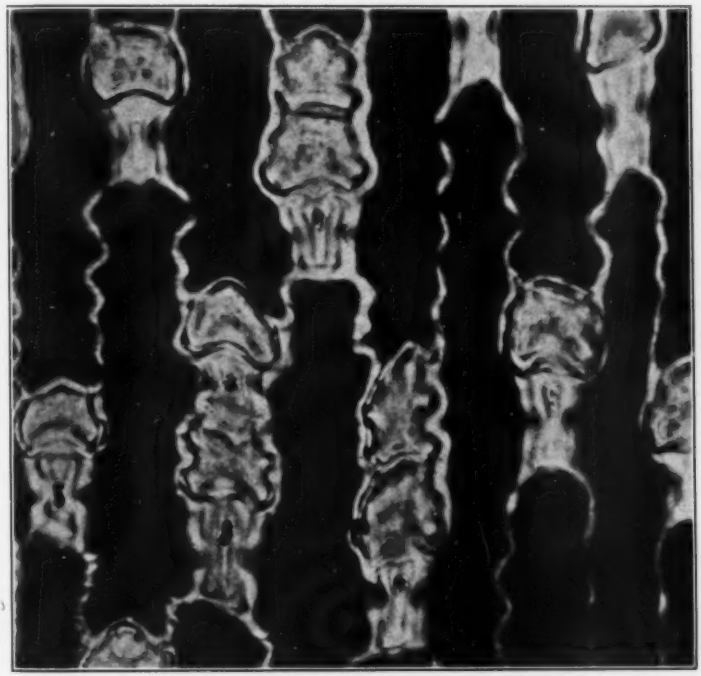


FIGURE 3.—Epidermis of Louisiana Purple. × 1,000

YELLOW CALEDONIA

The Yellow Caledonia variety is characterized by the partial or nearly complete absence of silica cells, a fact which makes it very easy to recognize, especially if one takes other characteristics into consideration. The pattern of the epidermis, viewed under low power, seems to vary. Sometimes the long cells are very regular in length and width; again the cells are variously shaped. Commonly, however, the long cells are rather narrow, there being 110 to 120 rows in a square millimeter, so that the width of the individual cell is on an average only 8.3 to 9 μ. The number of short-cell groups is about 800 in a square millimeter; the cells are trapezoidal, often pointed and hairlike. The cork cells are found singly or in groups, sometimes in pairs

with one member a silica cell. In general, however, silica cells are few in number. With low power one notices longitudinally disposed rows of epidermal cells which possess thicker and more strongly silicified walls than the neighboring rows. The lumen of the cells often contains silica. Stomata are also quite rare, since never more than one is observed in a microscopic field.

P. O. J. 2714

The pattern of the epidermis of P. O. J. 2714 is very uniform and the cells themselves are regular. There are about 94 rows of long cells in a square millimeter, so that their average width is 10.6 μ. The number of short-cell groups is 816 to 916, which closely agrees with the previously described varieties. The long cells are apt to vary considerably in width. Since their walls are of medium thickness or less, these cells have a comparatively broad lumen and appear to be wider than they actually are. A comparatively large number of the long cells are filled with crystal sand. The short-cell groups contain in most cases only one type of cell, the cork cell. Because of the absence of the silica cell, the cork cell very often stands alone; occasionally the cork cells appear in twos and even threes. When solitary the cork cell is rectangular or trapezoidal; in association with other cork cells it is shorter and sometimes pointed. Stomata are rather common, although their distribution is erratic. One often counts as many as 10 or more in a microscopic field.

FIGURE 4.—Epidermis of P. O. J. 213. × 330

U. S. 759

U. S. 759, which is relatively thin stalked as compared with the varieties described above, possesses an epidermis made up of comparatively large cells. The epidermal pattern is remarkably uniform. There are 72 rows of long cells in a square millimeter, so that their average width is 13.6 μ. The group of short cells is also greatly reduced in number, there being only 428 to 440 in a square millimeter as contrasted with 1,000 or more in D–74. The long cells are very uniform and possess transverse end walls. The calcium oxalate cells occur singly or in groups and are readily recognized by their glistening contents. The composition of the short-cell groups is similar to that in the preceding variety. The short cells appear in pairs (fig. 6) or the silica cell is wanting. The distribution of the stomata is erratic. Sometimes two are counted in a microscopic field; sometimes not even one.

P. O. J. 36

The P. O. J. seedlings that have Chunnee as the male parent are characterized by an epidermis in which the elongated or pointed cork cells are conspicuous and in which the epidermal pattern is more or less irregular.

The epidermis of P. O. J. 36 is quite irregular. The long cells appear narrow, more or less curved, and the end walls are often pointed. There are 74 rows of long cells in a square millimeter, their average width being 13.54 μ. The number of short-cell groups varies greatly and is difficult to estimate. The cork cells are quite irregular, often pointed and hairlike. The distribution of stomata is as irregular as in the other varieties. There are on an average 5 in a microscopic field, though as many as 12 have been counted.

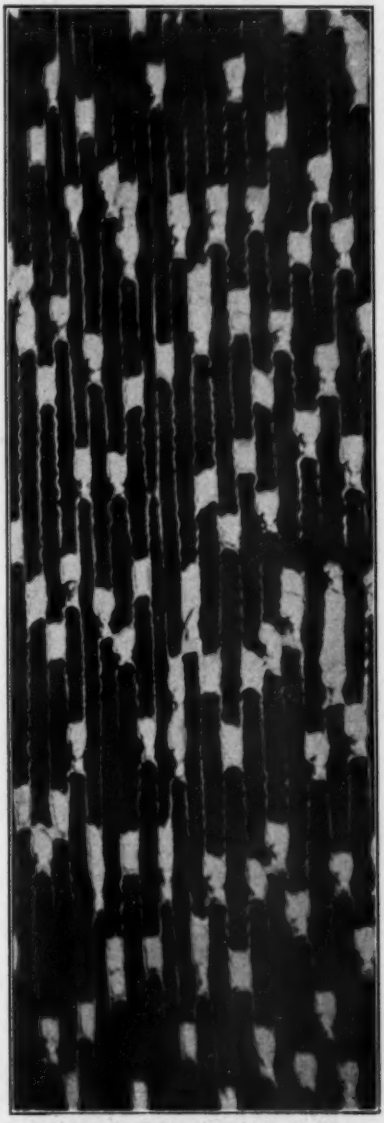


FIGURE 5.—Epidermis of D-74. × 330

P. O. J. 213

The epidermis of P. O. J. 213 is commonly very irregular and contains many elongated or hairlike cork cells. (Fig. 4.) The long cells have oblique or transverse end walls and are often of considerable length. They appear quite narrow, but actual counts show that there are on an average 89 rows of long cells in a square millimeter, so that their average width is 11.25 μ. The short cells appear in groups of 10 or more, one member of the group a silica cell. While the silica cells have the normal rectangular form characteristic of all varieties, the cork cells are variously shaped; and, since many of them are considerably elongated, the entire area they cover is at least equal to that covered by the ordinary epidermal or long cells. The actual number of short-cell groups in a square millimeter is approximately 372. Stomata are rare, usually less than one in a microscopic field.

P. O. J. 234

Compared to the variety just described, which it resembles greatly, P. O. J. 234 possesses a much more regular epidermis. The long cells are uniform and commonly have rectangular cross walls. There are 80 rows in a square millimeter, so that the average width of the cell

is 12.5 μ. The number of short-cell groups is greater than in the former variety, there being between 376 and 484 in a square millimeter. The groups of short cells are made up of both cork cells and silica cells. The cork cells, though of somewhat variable form, are much less conspicuous than those of P. O. J. 213. The cells are more or less rectangular, though there is a tendency for them to be triangular and even hairlike. Stomata, however, are more abundant, there being on an average four in a microscopic field.

KASSOER

The epidermal pattern in the Kassoer variety also is quite regular. (Fig. 7.) The long cells are relatively short, but sometimes much

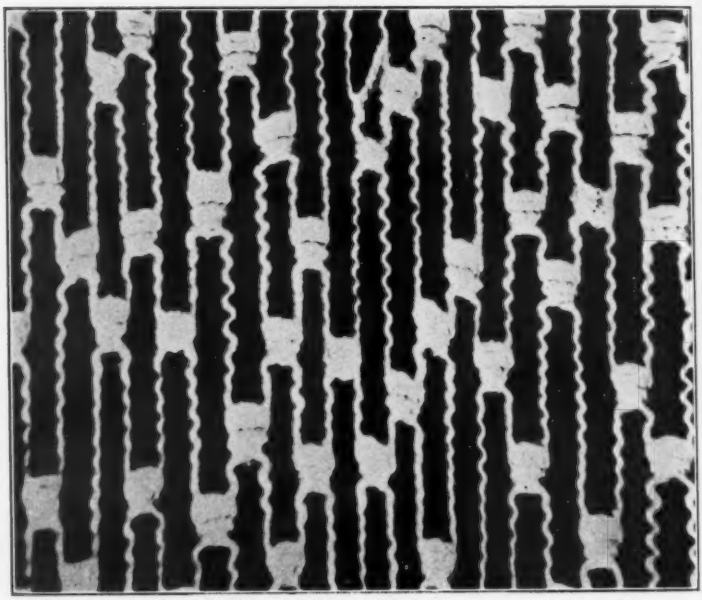


FIGURE 6.—Epidermis of U. S. 759. × 330

elongated; their end walls are typically rectangular but occasionally pointed. There are on an average 75 rows of long cells in a square millimeter, so that the average width of the long cells is 3.3 μ. The short cells are in groups of two or more, but the silica cell is occasionally wanting, so that the cork cells are solitary. Occasionally a silica cell stands alone between the long epidermal cells. The number of short-cell groups in a square millimeter varies from 364 to 480. The shape of the cork cell is commonly broadly reniform; occasionally the cells are rectangular, more often pointed and hairlike. The distribution of stomata is erratic, there being on an average one or two in a microscopic field.

U. S. 663

The epidermis of variety U. S. 663 appears very regular. (Fig. 8.) The long cells are uniform and have straight, sometimes pointed end

walls. There are 80 rows of long cells in a square millimeter, so that their average width is 12.5 μ. The number of short-cell groups varies, there being from 480 to 540 in a square millimeter. The cork cells are reniform, sometimes rectangular, sometimes pointed, and occasionally hairlike. The short cells occur in pairs; often, however, the silica cell is wanting. There is commonly one but sometimes as many as five stomata in a microscopic field.

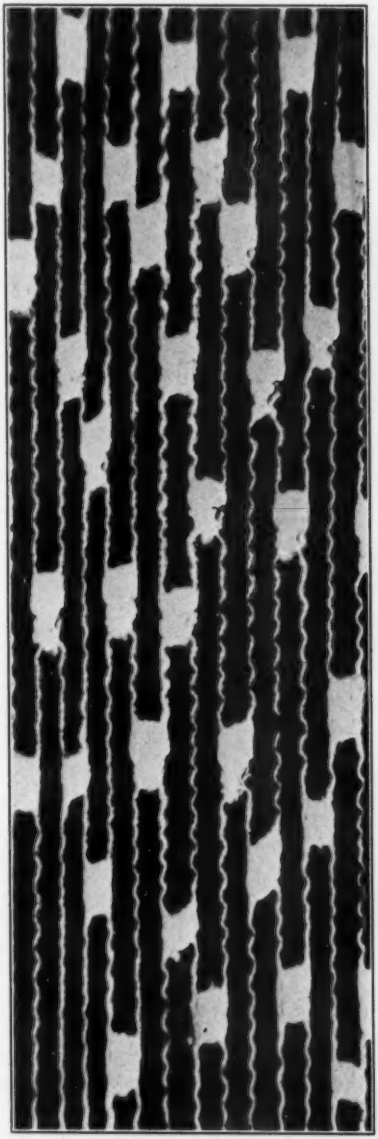


FIGURE 7.—Epidermis of Kassoer. × 330

U. S. 833

The epidermis of U. S. 833 resembles that of U. S. 663, except that the long cells are relatively short. The pattern is very uniform. There are 75 rows of long cells in a square millimeter, so that their average width is 13.3 μ. The number of short-cell groups is very uniform, there being on an average 656 in a square millimeter. The cork cells are usually reniform, occasionally square or pointed, sometimes hairlike. The short cells occur in pairs with one member a cork cell, the other a silica cell. Stomata are numerous, with as many as 15 in a microscopic field.

CAYANA

The pattern of Cayana is fairly uniform. (Fig. 2.) The long cells appear comparatively broad and short, but often they are quite elongated. Their end walls are usually rectangular. There are 75 rows of long cells in a square millimeter, so that their average width is 13.6 μ. The number of short-cell groups averages 644 in a square millimeter. The cork cells are uniform, sometimes rectangular, sometimes pointed. They are usually joined to silica cells, but the latter may occasionally be wanting so that the cork cells stand alone. Stomata are rare, but occasionally four to six are counted in a microscopic field.

CHUNNEE

The epidermis of the Chunnee variety is composed of small cells with an average diameter of 7.6 μ. It has, however, only 504 groups of small cells in a square millimeter. It differs in this respect from variety D–74, which, besides having narrow epidermal cells, has also a large number (over 1,000) of groups of small cells. The pattern of the epidermis is very uniform, with silica and cork cells in typical pairs. Stomata are infrequent.

SACCHARUM SPONTANEUM

Although *Saccharum spontaneum*, like Chunnee, is a thin and fibrous cane, the epidermis is made up of large cells (fig. 9, A) with an average width of 12.5 μ. The number of short-cell groups is small, rarely exceeding 400 square millimeters. Silica cells are usually wanting, but stomata (fig. 9, B) are comparatively abundant.

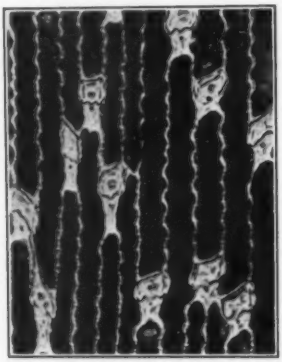


FIGURE 8.—Epidermis of U. S. 663. × 500

DISCUSSION

The variations in epidermal structure, as shown in Table 1, fall into two classes—qualitative and quantitative. Qualitative differences consist in the absence of silica cells and of elongated or hair-like cork cells. In the last analysis, however, these also are quantitative variations, since there is rarely a complete absence of either type; but even the partial absence of silica cells presents a picture so striking that some varieties could be recognized by this character alone. This is true, to a lesser degree, of the elongated or pointed cork cells also. There are varieties in which they are practically absent, while in others, especially P. O. J. 213 and 36, they are so abundant that one would experience little difficulty in identifying the variety. Among quantitative variations are the width and length of the long epidermal cells, the number of short-cell groups in a unit area, and the distribution of the stomata.

The variation in the width of the epidermal cells often aids in the separation of varieties when other diagnostic characters fail. It is important, however, to estimate correctly the average width of the cells. This may be difficult in varieties which have an irregular epidermis, as for example, P. O. J. 36. One can calculate the average width of the long cells by counting the number of rows in the microscopic field and dividing the diameter of the field by that number; more accurate counts, however, are obtained by projecting the stained preparation on a white screen. The thickness of the walls of the epidermal cells is more or less uniform. As a rule, a narrow-celled epidermis, as for example in D–74, has comparatively thin walls. The type of undulation of the walls also varies in the different varieties, as can easily be seen in the accompanying figures.

A certain ratio exists between long-cell and short-cell areas which can be calculated by determining the number of groups of short cells

in a unit area. It must be borne in mind, however, that in varieties with single-cell groups, that is, where the silica cells are wanting, the area of short cells will be less, while in varieties with a large number of elongated or hairlike cork cells this area will be greater than the calculated value.

The distribution of stomata, although erratic, offers a valuable diagnostic character. P. O. J. 213 and P. O. J. 234 are much alike in

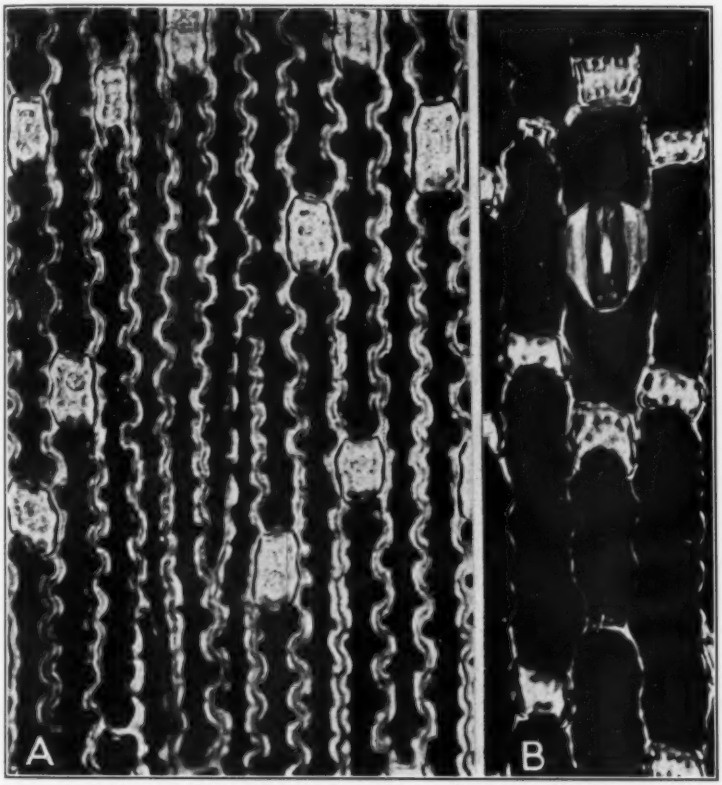


FIGURE 9.—Epidermis of *Saccharum spontaneum:* A, Showing solitary cork cells. × 500. B, Showing a stoma and pitting of the cork cells. × 1,000

the appearance of the epidermis; the former, however, has only one or two stomata per microscopic field, while the latter has from four to six.

In order to trace group relationships and analyze the parental influence in the various crosses, it would be necessary to examine the epidermis of a large number of canes, especially the early parent types. It would be of interest to trace the development of the elongated or hairlike cork cells back to the ancestral forms. All P. O. J. canes examined, with the exception of P. O. J. 2714, possess this type of cell. The male parent of these varieties is Chunnee and the female parents are Louisiana Purple and Gestrept Preanger, respectively. Neither

of the parents possesses the elongated cork cells, and it might be inferred that this character belongs to some earlier ancestor. In Kassoer and the U. S. seedlings these pointed cork cells are practically absent. At the same time these varieties exhibit in a greater or less degree a suppression of the silica cells, causing many of the short-cell groups to be composed of solitary cork cells. These two characters—that is, absence of elongated cork cells and suppression of the silica cells—are most significant in the epidermis of *Saccharum spontaneum*, a distant male ancestor of these varieties. The distribution of stomata is another character that defies a ready analysis. There appears to be no correlation between the number of stomata and the size of the epidermal cells. In D–74, which is the smallest-celled variety studied, the number of stomata is comparatively large, while in Chunnee, which also possesses small cells, stomata are rare.

TABLE 1.—*Epidermal characters useful in separating cane varieties*

Variety	Average width of cells μ	Number of short-cell groups per square millimeter	Pointed elongated cork cells	Solitary cork cells	Number of stomata per microscopic field [a]
Louisiana Purple	10.0	±928	Absent	Scarce	1–2.
D–74	7.7	±1,112	do	Abundant	14.
Yellow Caledonia	9.1	896–780	Present	do	1 or less.
P. O. J.:					
2714	10.6	816–916	Absent	do	10.
36	13.54	±528	Present	Absent	12.
213	11.25	±372	do	do	1.
234	12.5	376–484	do	do	4.
Kassoer	13.3	364–480	Scarce	Scarce	1–2.
U. S.:					
663	12.5	480–540	Absent	Abundant	1.
833	13.3	±656	do	Absent	15.
759	13.6	428–440	do	Abundant in places	2.
Cayana	13.6	±644	do	Scarce	4–6.
Saccharum spontaneum	12.5	±400	do	Abundant	
Chunnee	7.6	±504	do	Absent	

[a] Covered by a 16-mm. objective and a No. ×6 ocular, or an area of 2.4 sq. mm.

The following key affords, in a restricted sense, a means of identifying sugarcane varieties and hybrids of *Saccharum officinarum* and of tracing group relationships. It is as yet limited in its scope because of the restricted number of varieties studied, but its field of usefulness may be increased by extending the investigation to all important varieties.

KEY

I. Epidermis small-celled. Number of short-cell groups exceeding 20 per microscopic field (2-mm. oil-immersion objective and No. 6 eyepiece covering an area of 0.025 sq. mm.). Average width of elongated epidermal cells mostly below 10 μ.
  1. Elongated pointed cork cells numerous ..... *Yellow Caledonia.*
  2. Elongated cork cells wanting or few in number.
    A. Cork cells often solitary ..... *P. O. J. 2714.*
    B. Short-cell groups usually in pairs or double pairs with one member of the pair a cork cell, the other a silica cell.
      a. Stomata numerous ..... *D–74.*
      b. Stomata scarce ..... *Louisiana Purple.*

II. Epidermis large celled. Number of short-cell groups 16 or less per microscopic field.
1. Elongated cork cells more or less conspicuous.
A. Stomata rare ---------- *P. O. J. 213.*
B. Stomata numerous.
a. Elongated cork cells abundant; 5–12 stomata per field (16-mm. objective, No. 6 eyepiece) ------ *P. O. J. 36.*
b. Elongated cork cells less conspicuous; stomata 4–6 per field ---------- *P. O. J. 234.*
2. Elongated cork cells few in number or wanting.
A. Stomata scarce, rarely more than one per microscopic field.
a. Solitary cork cells numerous ------- *U. S. 663.*
b. Solitary cork cells less evident.
(1) Distribution regular ---------- *Kassoer.*
(2) Distribution erratic ---------- *U. S. 759.*
B. Stomata numerous ---------- *U. S. 833.*

SUMMARY

The stem epidermis of sugarcane can be removed for study by the use of potassium chlorate and nitric acid, and its component structures differentiated by staining with chloroiodide of zinc.

The epidermis is composed of ordinary or "long" cells and of "short" cells. The latter occur commonly in pairs with one member a cork cell, the other a silica cell. The silica cell is biscuit-shaped, varies little in size and form, and may be wanting. The cork cell is reniform, rectangular, trapezoidal, or elongated like a hair. The occurrence of elongated cork cells is more or less restricted to certain varieties. Stomata are very rare in some varieties, less so in others, and always erratic in distribution.

Since the structure of the epidermis is characteristic of the variety and since its composition appears to be little influenced by environmental factors, it is useful in identifying varieties.

CHROMOSOME BEHAVIOR AND POLLEN PRODUCTION IN THE POTATO [1]

By A. E. LONGLEY, *Associate Botanist, Biophysical Laboratory,* and C. F. CLARK, *Associate Horticulturist, Office of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The propagation of potatoes from seeds is seldom resorted to by the commercial grower, asexual reproduction by means of tuber cuttings being the universal practice. Seeds, however, have been much used by potato breeders as a means of producing new varietes because of the wide range of variability which has been found to occur in the progenies produced in this manner.

It is generally known that seed production in the potato is the exception rather than the rule. Numerous investigations have been made to determine the reason for the nonfruitfulness of potatoes. East (*4*),[2] Dorsey (*3*), and more recently Breeze (*1*) found that there is a great variation in the amount of viable pollen produced by different varieties of potato and that in many of them there is a large percentage of defective pollen. Stout and Clark (*18*) made an extensive study of pollen conditions both in wild potato species and in cultivated varieties. In the wild species they generally found abundant viable pollen, while among cultivated varieties they found very few that produced an appreciable amount.

Pollen studies show the plant breeder that the lack of viable pollen is largely responsible for the nonproduction of seed in potatoes, but he is still in the dark as to the cause of the high percentage of abortive pollen. For this reason the writers undertook to investigate the pollen mother-cell development of the cultivated potatoes and their allies. It was hoped that such a study would (1) give a more complete picture of the sterility problems that confront the potato breeder, and possibly indicate why cultivated potatoes differ so markedly from wild species in their ability to produce viable pollen; and (2) show whether varietal differences are associated with chromosomal differences and whether anything in the number or behavior of the chromosomes would give a clue to the ancestry of the cultivated potato. It was believed that information on these points might be useful to potato breeders.

MATERIAL AND METHODS

This study treats of the number and meiotic behavior of chromosomes in tuber-bearing forms of Solanum. These forms came originally from widely different sources and belong to the section Tuberarium of the genus Solanum. Cytological material was collected from 2 unnamed seedlings and 38 commercial varieties belonging to the group known as *Solanum tuberosum* L. and from 11 wild forms referred to 10 distinct species.

[1] Received for publication July 24, 1930; issued December, 1930.
[2] Reference is made by number (italic) to Literature Cited, p. 887.

The buds from which material showing the developing pollen mother cells was taken were collected, with few exceptions, at the Aroostook Farm of the Maine Agricultural Experiment Station at Presque Isle, Me., in the plots grown under the supervision of the Office of Horticultural Crops and Diseases of the Bureau of Plant Industry, United States Department of Agriculture.

Material was collected during the latter part of July in the years 1928 and 1929. The collections were made during the warmer part of the day, and acetocarmine preparations were made at once from fresh material. Other material was killed in Carnoy's fluid and brought to the laboratory at Washington for future study.

The preparations in acetocarmine made from fresh material were most satisfactory for studying chromosome numbers. The killed and fixed material was useful, however, in studying the general type of divisions and the character of the tetrads typical of the different varieties.

The collecting of material in two different seasons made it possible to compare the behavior of a variety grown in slightly different environments.

Camera lucida drawings and photomicrographs were made of characteristic figures observed in the preparations. A Leitz 1/16 apochromatic oil-immersion lens and × 8 and × 15 oculars were used throughout these studies.

CHROMOSOMES IN WILD SPECIES OF SOLANUM

Plants are frequently found in which the somatic chromosome complement is made up of more than two intermingled sets. Such plants are spoken of as being polyploid. The term "polyploid" is not restricted to plants known to have more than two homologous chromosome sets, but is frequently applied to forms in which the chromosome number alone is the only suggestion of the presence of several sets of chromosomes. It is in this sense that the present writers use the terms "triploid," "tetraploid," etc.

Eleven wild Solanum forms shown in Table 1 have their diploid chromosome numbers in definite multiples of 12. Since no Solanum species has been found with less than 12 as its haploid or less than 24 as its diploid chromosome number, these are considered the basic chromosome numbers for the genus, and the species with 36, 48, and 72 somatic chromosomes are spoken of as being triploid, tetraploid, and hexaploid, respectively.

TABLE 1.—*Chromosome numbers in wild forms of potato*

Name	Chromosome class	Haploid chromosome number	Investigator
Solanum caldasii	Diploid	12	Vilmorin and Simonet (*20*, *21*).
Solanum caldasii glabrescens	do	12	Present writers.
Solanum chacoense	do	12	Smith (*17*); present writers.
Solanum jamesii	do	12	Vilmorin and Simonet (*20*, *21*); Smith (*17*); present writers.
Solanum polyadenium	do	12	Present writers.
Solanum cardiophyllum f. coyoacanum	Triploid	18	Do.
Solanum commersonii	do	18	Do.
Solanum ajuscoense	Tetraploid	24	Do.
Solanum antipovichi	do	24	Do.
Solanum fendleri	do	24	Smith (*17*); present writers.
Solanum demissum	Hexaploid	36	Do.

Chromosome counting is frequently a difficult problem. For this reason gametic cells, in which the reduced or haploid chromosome number is found, are most often used by cytological investigators. The present study of chromosome number has been for the most part confined to the developing pollen mother cells.

The four wild forms *Solanum caldasii glabrescens* Dunal, *S. chacoense* Bitter, *S. jamesii* Torr., and *S. polyadenium* Greenm. have 12 as their haploid chromosome number. A and B of Figure 1 show first-division metaphases of *S. caldasii glabrescens* and *S. polyadenium*, respectively. A second-division metaphase of *S. chacoense* is illustrated in Figure 1, C. All four species show regular pairing and distribution of chromosomes during the meiotic phases of their pollen mother-cell development.

The chromosome behavior during the reduction divisions of F_1 and F_2 hybrids between the two species *Solanum caldasii glabrescens* and *S. chacoense* was studied. In both generations there was a regular pairing of the chromosomes, and a large percentage of the chromosome

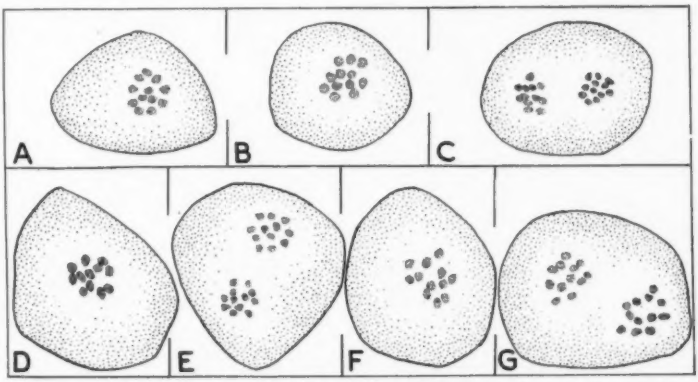


FIGURE 1.—Pollen mother cells from diploid species and hybrids: A, B, D, and F, First-division metaphase in *Solanum caldasii glabrescens*, *S. polyadenium*, *S. tuberosum* S. A. 336, and *S. caldasii glabrescens* × *S. chacoense*, respectively. C, E, and G, Second-division metaphases of *S. chacoense*, *S. tuberosum* S. A. 336, and *S. caldasii glabrescens* × *S. chacoense*, respectively. × 2,000

tetrads were normal in appearance. Figure 1, F, shows a first-division metaphase from an F_1 hybrid, in which 12 bivalent chromosomes are arranged on the plate. Figure 1, G, is a second-division metaphase from an F_2 hybrid; in both plates the 12 chromosomes are clearly shown.

Two wild forms, *Solanum cardiophyllum* f. *coyoacanum* Bukasov and *S. commersonii* Dunal, were found to be triploid, with 18 as their haploid chromosome number. In any preparation from either of these species there were very few cells that showed a regular pairing of all chromosome units. The number of paired chromosomes varied from 12 to 18, with a corresponding variation in the number of univalents. Figure 2, A, shows a first-division metaphase of *S. cardiophyllum* f. *coyoacanum* with 8 univalents and 14 bivalents; B shows a cell of *S. commersonii* with 4 univalents and 16 bivalents; and C is a second-division metaphase of the latter species showing unequal numbers of chromosomes in the two plates and two extruded chromosomes.

The chromosome behavior of both of these forms is that usually resulting when two sets of incompatible chromosomes, or when two unequal chromosome sets, attempt to pair in the early phases of the first division of a hybrid plant. It is almost identical with the meiotic behavior of the artificially produced triploid hybrid described in the following paragraph.

Material from an F_1 hybrid between *Solanum fendleri* with 24 chromosomes and *S. chacoense* with 12 gave a chromosome behavior typical of a plant hybrid combining two unequal sets of chromosomes. Figure 2, D and E, are two first-division metaphases showing both paired and unpaired chromosomes. The bivalent chromosomes behave normally, but the univalents are distributed undivided at random in the first division, and frequently a few fail to be included in the two daughter nuclei. Figure 2, F, shows a second-division metaphase with 18 chromosomes on each plate. Many cells, however,

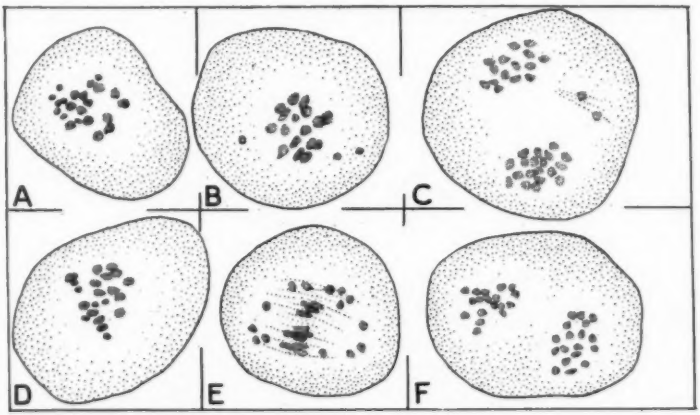


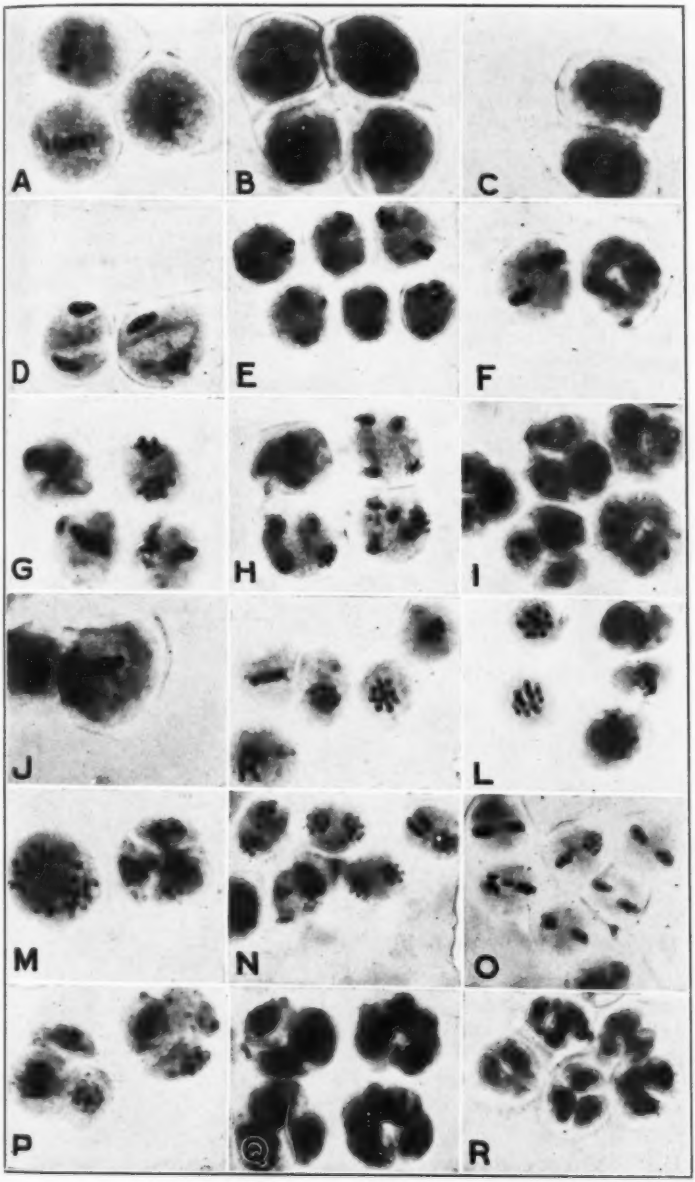
FIGURE 2.—Pollen mother cells from triploid forms and hybrids. A and B, First-division metaphases in *Solanum cardiophyllum* f. *coyoacanum* and *S. commersonii*, respectively. D and E, First-division metaphases in *S. fendleri* × *S. chacoense*, face view and side view, respectively. C and F, Second-division metaphases in *S. commersonii*, showing two chromosomes in the cytoplasm, and *S. fendleri* × *S. chacoense*, respectively. × 2,000

at this phase show unequal numbers on the two plates, and later phases frequently show smaller chromosome groups in addition to the four major groups.

Three wild species, *Solanum fendleri* Gray, *S. ajuscoense* Bukasov, and *S. antipovichi* Bukasov, were found to have twice as many chromosomes as the diploid species. A, B, and D of Figure 3 show first-division

EXPLANATORY LEGEND FOR PLATE 1

A and B.—First-division metaphases in Prince Albert and seedling No. 43752, respectively.
C and D.—Interkineses in Keeper and Prince Albert, respectively.
E.—Second-division metaphases in Keeper.
F and G.—Second-division metaphases in McCormick.
H.—Second-division anaphases in Keeper.
I.—Tetrads in seedling No. 43752.
J, K, and L.—First-division metaphases in *Solanum demissum*, *S. tuberosum* S. A. 302, and *S. caldasii glabrescens* × *S. chacoense*, respectively.
M.—First-division anaphase in *S. antipovichi*.
N and O.—Second-division metaphases in *S. caldasii glabrescens* × *S. chacoense* and *S. tuberosum* S. A. 302, respectively.
P, Q, and R.—Tetrads in *S. antipovichi*, *S. demissum*, and *S. tuberosum* S. A. 302, respectively.



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metaphases of these three species, in which 24 chromosomes may be counted. There was very little difficulty in obtaining good figures of both the reduction divisions in all three species, although *S. ajuscoense* showed considerable irregularity. Figure 3, C, illustrates a regular second-division metaphase of *S. ajuscoense;* the lower plate shows a side view, the upper plate a face view; in the latter the 24 chromosomes stand out distinctly.

Only one species, *Solanum demissum* Lindl., was found to have 36 as its haploid chromosome number. Figure 3, E, shows a first-division metaphase of a pollen mother cell from this form, in which the number is clearly distinguishable. The meiotic behavior of the chromosomes is regular and leads to the production of normal pollen tetrads. Smith (*17*) has found the same number for this species and reports nothing abnormal in its chromosome behavior.

Plate 1, J–R, is a series of photomicrographs illustrating the character of the first and second reduction-division phases in a group of regular-behaving potatoes, most of which are from the foregoing wild

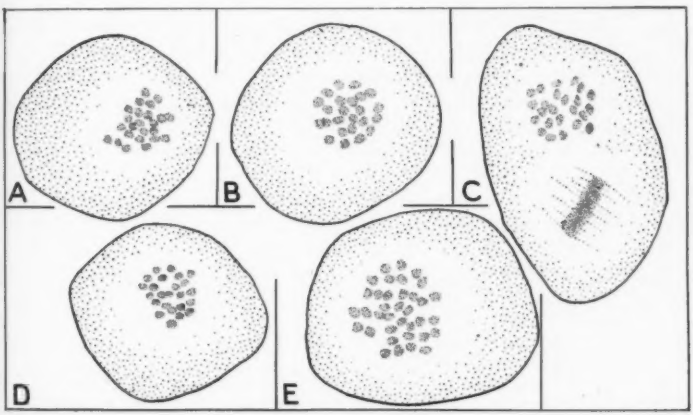


FIGURE 3.—Pollen mother cells from tetraploid and hexaploid species. A, B, D, and E, First-division metaphases in *Solanum antipovichi*, *S. ajuscoense*, *S. fendleri*, and *S. demissum*, respectively. C, Second-division metaphase in *S. ajuscoense*. × 2,000

species. J is a side view of a first-division metaphase of *Solanum demissum* in which the chromosomes are lying uniformly on the plate; L shows both face and side views of first-division metaphases of *S. caldasii glabrescens* × *S. chacoense* in which the chromosome pairing is regular. M shows a first-division anaphase and a 4-celled tetrad of *S. antipovichi*. N shows second-division metaphases and anaphases of *S. caldasii glabrescens* × *S. chacoense* which give no indication of irregularities in the second division. P and Q show tetrads of *S. antipovichi* and *S. demissum*, respectively. The chromosome behavior of any of these species is seldom irregular, and the tetrads usually appear normal.

This study of chromosomes in wild species of Solanum has shown that forms with an even multiple of the basic chromosome number, except possibly *S. ajuscoense*, have a regular meiotic behavior resulting in the production of 4-celled tetrads and that each cell of the

tetrad receives the same number of chromosomes. The chromosome behavior in the two triploid forms, however, suggests that they are hybrids in which two unequal chromosome sets or three chromosome sets have been brought together. The presence of univalent chromosomes results in an abnormal distribution of chromosomes to the daughter cells during pollen formation. The chromosome behavior of the two triploid forms is very similar to that described for the *S. fendleri* × *S. chacoense* hybrid, in which the chromosome sets of the two parents were unequal (possibly the female parent had two sets and the male one), and causes abnormal meioses typical of a hybrid of this type.

The second hybrid, *Solanum caldasii glabrescens* × *S. chacoense*, in which two equal chromosome sets were combined, showed nothing of an incompatible nature when the chromosomes of the two distinct species were brought together during meioses of the developing pollen mother cells.

CHROMOSOMES IN CULTIVATED POTATOES

Determinations of the chromosome number in 40 cultivated forms (Table 2) of *Solanum tuberosum* have shown that all except three yellow-fleshed varieties from South America have 24 as their gametic chromosome number. A study of the meiotic phases of these tetraploid varieties, however, show that very few of them have a regular chromosome behavior during pollen formation.

TABLE 2.—*Cell characteristics associated with pollen sterility in cultivated potatoes (Solanum tuberosum)*

Year	Variety		Distribution of pollen mother cells producing the following groups of cells—						4-cell groups		Pollen classes [a]	Chromosomes	
	Field No.	Name	2	3	4	5	6 or more	Total	Percentage	Standard error		Behavior	Number
1928	302	S. A.[b] Yellow Flesh			165	10	2	177	93.2	1.87		Regular	12
1928	336	do										do	12
1928	345	do										do	12
1928	46	Garnet Chili	4	3	90	12	2	111	81.1	3.72	+2	Group 1[c]	24
1928	35	Keeper		2	92	18	1	113	81.4	3.66	1	do	24
1929	41	Never Rot	6	4	91	4	2	107	85.0	3.45	2	do	24
1928	85	Prince Albert	1		85	19	4	109	78.0	3.96	3	do	24
1929	40	do			80	10	6	96	83.3	3.81	3	do	24
1929		Seedling No. 43986	2		120	5		127	94.5	2.02	1	do	24
1929		Seedling No. 43225	4		122			126	96.8	1.57	1	do	24
1928		McCormick	41	9	180	6	1	237	75.9	2.78	1	do	24
1929		do	33	2	80			115	69.6	4.29	1	Group 2	24
1929		do	39	5	90			134	67.2	4.05	1	do	24
1929		do	54	4	72			130	55.4	4.36	1	do	24
1929		do	81	2	50			133	37.6	4.20	1	do	24
1928		Green Mountain	46	12	43			101	42.6	4.92	2	do	24
1928		do	80	9	42			131	32.1	4.08	2	do	24
1928		do	55	9	92			156	59.0	3.94	2	do	24
1928		do	78	7	43			128	33.6	4.17	2	do	24
1929	23	do	68	8				76	0		2	do	24
1929	23	do	108	12	1			121	.83	.81	2	do	24
1929	23	do	99	18	8			125	6.4	2.19	2	do	24
1929	23	do	96	37	17			150	11.3	2.58	2	do	24
1929	A	Irish Cobbler	52	34	21			107	19.6	3.84	3	do	24
1929	A	do	53	17	60			130	46.2	4.37	3	do	24
1928	45p	Peachblow	9		99	6		114	86.8	3.16	3	do	24
1928	45p	do	16	5	141	1		163	86.5	2.63	3	do	24
1928	45p	do	4	2	152			158	96.2	1.52	3	do	24
1929	26	do	21	29	53			103	51.5	4.95	3	do	24
1929	26	do	57	15	62			134	46.3	4.30	3	do	24
1929	26	do	33	5	29			67	43.3	6.05	3	do	24
1929	26	do	42	14	48			104	46.2	4.88	3	do	24
1928	7	White Albino	48	19	38			105	36.2	4.68	3	do	24
1928	20	Beauty of Hebron	92	6				98	0		3	Group 3	24
1929	20	do	83	30	1			114	.88	.88	3	do	24
1928	12	American Giant	30	4				34	0		3	do	24
1929	21	do	99	2				101	0		3	do	24
1929		Blue Victor	53	16	11			80	13.8	3.86		do	24
1929	15	Early Sunrise, Buist's	45	97	19			161	11.8	2.54	−3	do	24

TABLE 2.—*Cell characteristics associated with pollen sterility in cultivated potatoes (Solanum tuberosum)*—Continued

Year	Variety		Distribution of pollen mother cells producing the following groups of cells—						4-cell groups		Pollen classes [a]	Chromosomes	
	Field No.	Name	2	3	4	5	6 or more	Total	Percentage	Standard error		Behavior	Number
1928	53	Dakota Red	90	6	1			97	1.03	.959	4	Group 3	24
1929	29	do	106	7				113	0		4	do	24
1928	11	Early Manistee	92	9	3			104	2.88	1.65	+4	do	24
1929	7	do	139	19	4			162	2.47	1.23	+4	do	24
1929	11	Early Ohio	120	9				129	0		3	do	24
1929	A	Early Rose	107	8				115	0		3	do	24
1928	56	Jersey Red Skin	100	7				107	0		3	do	24
1929		do	129	17				146	0		3	do	24
1929	4	Noroton Beauty	67	17				84	0		−3	do	24
1929		Peoples	102		2			104	1.92	1.33		do	24
1928	87	Peerless	117	2				119	0			do	24
1929		Peerless (Pearl)	115		1			116	.86	.87	−3	do	24
1929	31	Queen of the Valley	133	16				149	0		−3	do	24
1928	41	Russet Rural	55	26	2			83	2.41	1.68	−3	do	24
1929	24	do	100	9				109	0		−3	do	24
1928	9	Scotch Rose	107	28				135	0		−3	Groups 3 and 4	24
1929		do	86	17	3			106	2.83	1.60	−3	do	24
1929		Triumph	78	19	5			102	4.90	2.14	3	Group 3	24
1928	54	Adirondack	105	22				127	0		−3	Group 4	24
1929	30	do	63	13	1			77	1.30	1.29	−3	do	24
1928	61	Charles Downing	86	14	5			105	4.76	2.10	4	do	24
1929		do	91	8	2	1		102	1.96	1.37	4	do	24
1929	1	do	61	3	2			66	3.03	2.09	4	do	24
1929	39	Cowhorn	105					105	0		4	do	24
1929	38	McCullock	78	19	5			102	4.90	1.82	4	do	24
1928	48	Perfect Peachblow	118	12				130	0		+4	do	24
1928	59	Prolific	106	9				115	0		4	do	24
1929	8	do	156	4				160	0		4	do	24
1928	23	Pride of Multnomah	104					104	0		4		24
1928	40	Carman No. 1	207	11				218	0		3		24
1928	19	King of the Roses	107	8				115	0				24
1928	10	Maggie Murphy	102	13				115	0				24

[a] Pollen classes as defined by Stout and Clark (*18, p. 6-7*).
[b] South American.
[c] Groups as defined in text, p. 876.

The chromosomes of three yellow-fleshed varieties (Nos. 302, 336, and 345) were very easily counted. The 12 bivalent chromosomes were frequently seen arranged on the metaphase plate of the first reduction division. (Fig. 1, D, and pl. 1, K.) The chromosomes divide regularly in both reduction divisions of the developing pollen mother cells, and counts could also be made when the chromosomes were on the metaphase plates of the second division. (Fig. 1, E, and pl. 1, O.) It was exceptional to find univalent chromosomes at diakinesis, or unequal distribution of the chromosomes to the four nuclei of the pollen tetrad.

The chromosome behavior of the remaining 37 varieties was frequently so variable that chromosome counts were often made with difficulty. Whenever both univalent and bivalent chromosomes were present it was necessary to distinguish between the two types in order to make counts accurately. Because of the small size and the large number of chromosomes there was danger of making mistakes. Root-tip material from four varieties—McCormick, Green Mountain,

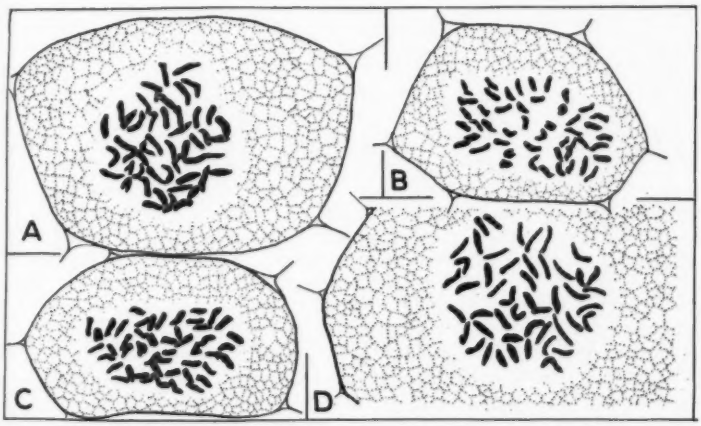


FIGURE 4.—Root-tip cells from four potato varieties, each showing 48 somatic chromosomes: A, Early Rose; B, Charles Downing; C, McCormick; D, Green Mountain. × 2,000

Early Rose, and Charles Downing—was prepared and somatic counts made. A to D of Figure 4 show representative cells from the four varieties, in which 48 somatic chromosomes are distinguishable.

Pollen mother-cell preparations of all varieties showed chromosome numbers that left little doubt that they should be assigned to the tetraploid group, and the somatic count of four representative varieties supports the conclusion that there is no variation in chromosome number in the commonly cultivated varieties of potatoes.

The meiotic behavior of the chromosomes in developing pollen mother cells in this tetraploid group of potatoes shows nothing of the constancy observed in the chromosome number, and abnormalities are so varied that each variety would require individual examination to picture accurately the varying methods of pollen formation characteristic of it. On the basis of variation in chromosome behavior, the tetraploid varieties have been separated into four groups, as fol-

lows, both for convenience and to emphasize the different types of irregularities.

GROUP 1.—Varieties in which there are frequently some unpaired chromosomes among the bivalents, but in which both the first and second reduction divisions usually occur.

GROUP 2.—Varieties in which there are frequently some unpaired chromosomes, with an occasional cell showing no pairing, and in which there are some cells that go through one division only.

GROUP 3.—Varieties in which pairing is variable and in which pollen mother cells rarely go beyond one division and a number of the cells show no pairing of chromosomes.

GROUP 4.—Varieties in which a large percentage of the cells show the somatic chromosome number and in which true meioses are frequently absent. The nucleus degenerates in the prophase or metaphase and the cell divides in an amitotic manner.

The seven varieties—Keeper, Garnet Chili, Never Rot, Prince Albert, two seedlings (Nos. 43225 and 43986, selected because of their fertility), and McCormick—fit best the conditions described for Group 1, although McCormick in 1929 came nearer the conditions described for Group 2.

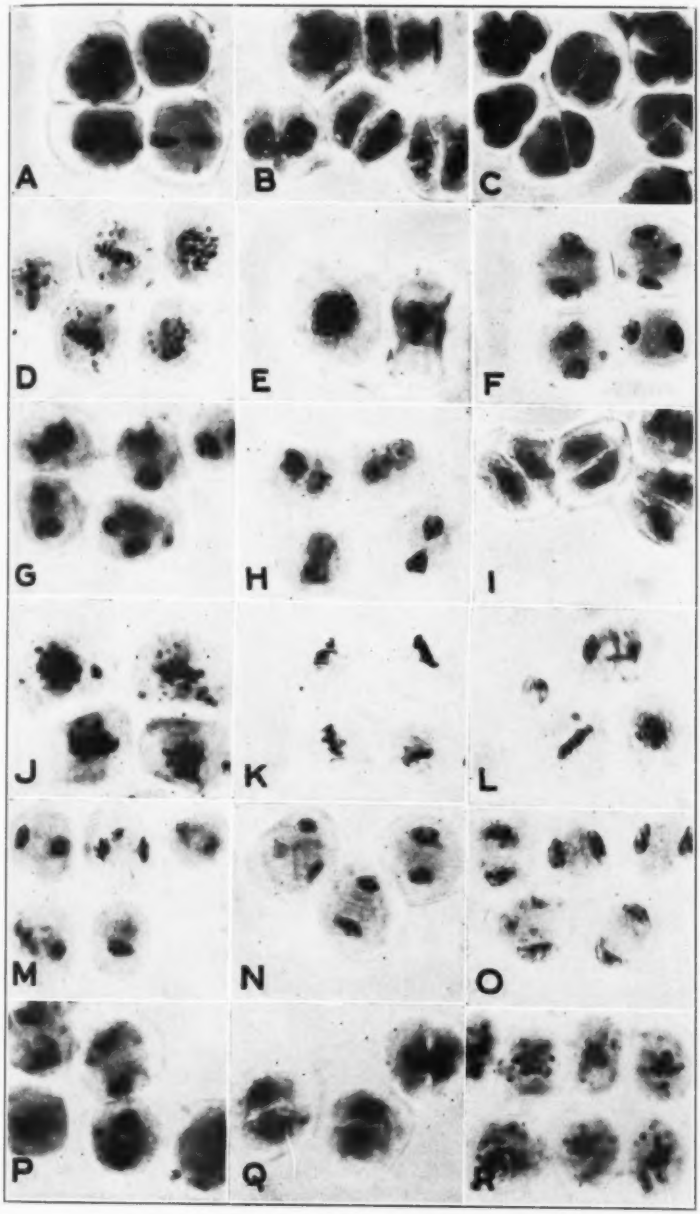
Each of the foregoing varieties gave suitable material from which chromosome counts could be made in metaphases of both the first and second reduction divisions. Figure 5, D, is a metaphase of the first-reduction division of McCormick, showing 24 bivalent chromosomes. E and F of Figure 5 are metaphases of the second division of Prince Albert and seedling No. 43225, respectively. In E both plates show 24 chromosomes, while F shows 24 chromosomes in one plate and an extruded chromosome in the cytoplasm which had failed to be included in the plate shown in side view.

Although a majority of the pollen mother cells go through their reduction phases normally, unpaired chromosomes are often seen in the early phases of the first reduction division, and if some fail to be included in the two daughter cells there will be more than the usual four cells produced from a single mother cell

Plate 1, A–I, is a series of photomicrographs that illustrate characteristic phases in chromosome distribution of plants belonging to this most regular group of tetraploid potato varieties. A and B are first-division metaphases of Prince Albert and seedling No. 43752, showing side views of the chromosomes on the plate. It is exceptional to find in either of these varieties univalents scattered on the spindle. C and D are interkineses of Keeper and Prince Albert, respectively, showing for each cell two equal-sized nuclei. E, F, and G are second-division metaphases of Keeper and McCormick—a phase seldom seen except in the seven varieties of this group. H shows second-division anaphases of Keeper, and I 4-celled tetrads of seedling No. 43752. It is difficult to photograph a tetrad with all four cells showing,

EXPLANATORY LEGEND FOR PLATE 2

A.—Second-division metaphases in Peachblow.
B and C.—Two-cell, 3-cell, and 4-cell pollen groups in Peachblow.
D.—Metaphases in Russet Rural.
E.—Metaphases in Blue Victor showing the regular plates in cells when all chromosomes are unpaired.
F.—Telophases in Russet Rural.
G and H.—Telophases in Peerless (Pearl).
I.—Two-cell pollen groups in Dakota Red.
J, K, and L.—Metaphases in Charles Downing, many of which show no pairing of chromosomes.
M, N, O, and P.—Telophases in Prolific, Charles Downing (N and O), and Cowhorn, respectively.
Q.—Two-cell pollen groups in Cowhorn.
R.—First-division metaphases in *Solanum fendleri* × *S. chacoense*.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE

as the fourth cell is usually in a different plane from that of the other three.

Four varieties—Green Mountain, Irish Cobbler, White Albino, and Peachblow—and also McCormick collected in 1929, fit the behavior described for Group 2.

From 25 to 75 per cent of the pollen mother cells of the above-listed varieties pass through both reduction divisions in a fairly regular manner, except for the presence of univalent chromosomes, which are distributed at random or extruded.

A and B of Figure 5 are first-division metaphases of Green Mountain and a variant of Green Mountain known as Giant Hill, respectively. The haploid number, 24, is shown, but it is exceptional to find a cell with all chromosomes paired. These cells, in which all chromosomes are paired, are pictured because they show best the

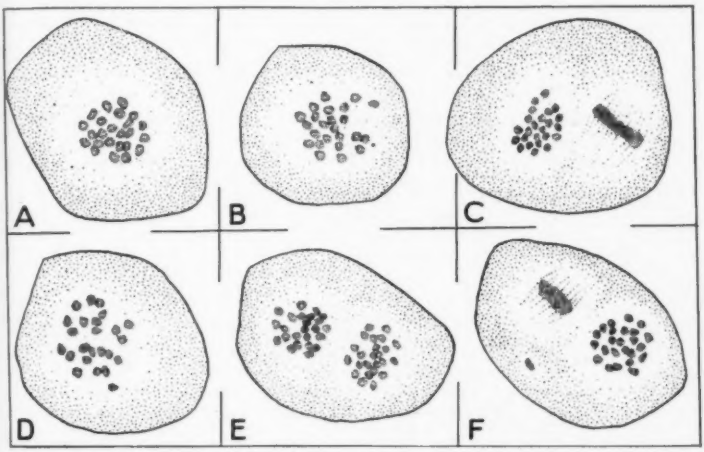


FIGURE 5.—Pollen mother cells from potato varieties: A, B, and D, First-division metaphases of Green Mountain, Green Mountain Giant Hill, and McCormick, respectively. C, E, and F, Second-division metaphases in Green Mountain Giant Hill, Prince Albert, and seedling No. 43225, respectively. ×2,000

true chromosome number for these varieties. A regular second-division metaphase of Green Mountain Giant Hill is illustrated in Figure 5, C.

The chromosome behavior that separates these varieties from those of Group 1 is the occasional presence of a cell showing no paired chromosomes and the frequent appearance of cells in which there is no second division, so that many pollen mother cells produce only two pollen grains. Occasionally, however, chromosomes are distributed into three groups, or even more, giving consequently three or more pollen grains from cells that never make more than one division.

Plate 2, A–C, shows three photomicrographs of Peachblow. A is a second-division metaphase—a phase that is found much less frequently than in varieties of Group 1. B and C show pollen groups of this variety. The number of cells from a mother cell is variable, and these photographs show 2-cell, 3-cell, and 4-cell pollen groups that have been produced by sister cells.

Group 3 includes the following 16 varieties, and in all of these the number of cells making both first and second divisions is less than 10 per cent and in most varieties almost zero:

American Giant.
Beauty of Hebron.
Blue Victor.
Dakota Red.
Early Manistee.
Early Ohio.
Early Rose.
Early Sunrise, Buist's.
Jersey Red Skin.
Noroton Beauty.
Peerless.
Peoples.
Queen of the Valley.
Russet Rural.
Scotch Rose.
Triumph.

Almost any preparation in which pollen mother cells of these varieties are found will contain some cells showing no chromosome pairing. These cells often afforded an opportunity to obtain chromosome counts in varieties in which regular pairing was seldom seen. In cells having both univalent and bivalent chromosomes it is difficult to count the chromosomes accurately, and the studies were made easier by the presence of cells showing no paired chromosomes. The number of cells containing the full somatic number varied in different varieties.

B and C of Figure 6 show two cells from a preparation of Beauty of Hebron, one having 48 small univalent chromosomes, the other 24

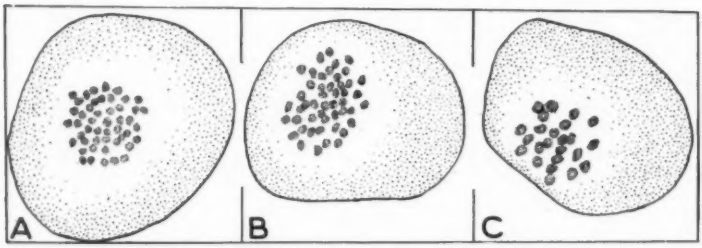


FIGURE 6.—Pollen mother cells from potato varieties: A, Metaphase of Prolific showing no chromosome pairing. B and C, First-division metaphases of Beauty of Hebron showing 48 univalent chromosomes and 24 bivalent chromosomes, respectively. × 2,000

larger bivalent chromosomes. Two similar cells of Peoples are shown in Figure 7, A and B. E and F of Figure 7 are cells of Blue Victor and Triumph, respectively, which have been crushed slightly to separate the chromosomes. They serve to compare the size of univalents and bivalents. In E there are 22 univalents that either failed to pair or have divided after pairing and 13 paired chromosomes, while in F there are 23 paired and 2 univalent chromosomes.

Cell division often follows immediately after a single division of the chromosome, and consequently a single pollen mother cell produces only two pollen grains, except when the chromosomes are distributed into more than two groups or when no division of the cell takes place, giving, consequently, from a single mother cell, one or three or more pollen grains.

In Plate 2, D–I, is presented a series of photomicrographs illustrating the characteristic procedure in pollen formation of varieties of Group 3. D shows metaphases of Russet Rural with paired chromosomes on the plate and univalents scattered on the spindle. E shows two cells of Blue Victor in which there was no chromosome pairing; the 48 univalent chromosomes form a regular plate very similar to

that seen in a somatic cell. F, G, and H show cells of Russet Rural and Peerless before cell division has taken place. Two or three nuclei produced from a single irregular nuclear division may be seen. I is a typical photograph, showing 2-celled pollen groups of Dakota Red that have resulted from a single division of the pollen mother cell.

The fourth group includes Charles Downing, Prolific, McCullock, Cowhorn, Perfect Peachblow, Russet Burbank, and Scotch Rose. Scotch Rose stands between Groups 3 and 4, showing characteristics sometimes of the one and sometimes of the other. This variety, therefore, is included in both groups.

The outstanding characteristic of a group of cells from a preparation of any of the foregoing varieties is the prevalence of cells having 48 unpaired chromosomes. One would be led to class these varieties as octoploid except for the presence of cells showing paired chromosomes, mixed with others showing no chromosome pairing. The

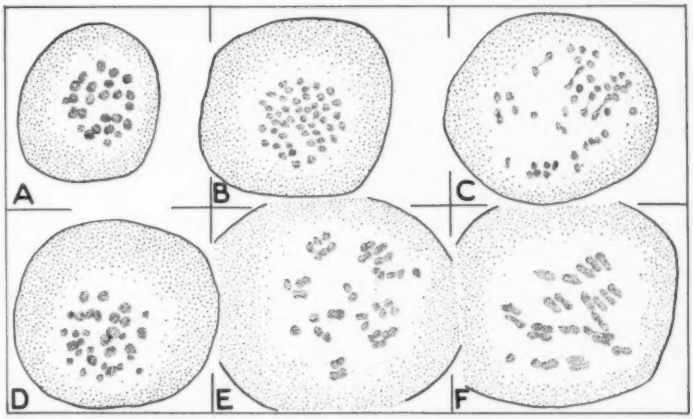


FIGURE 7.—Pollen mother cells from potato varieties: A, Metaphase from Peoples showing 22 bivalent and 4 univalent chromosomes; B, metaphase from Peoples showing 48 univalent chromosomes; C, late metaphase from Charles Downing; D, metaphase from McCullock showing 16 univalent and 16 bivalent chromosomes; E, late metaphase from Blue Victor showing univalent and bivalent chromosomes; F, metaphase from Triumph showing divided and dividing chromosomes. × 2,000

chromosomes in cells containing 48 chromosomes agree in size with the univalents in cells in which there is some pairing. The writers do not hesitate to place these 48-chromosome varieties in the tetraploid group, especially since one representative—the Charles Downing—was found to have 48 chromosomes in the root-tip cells.

Very little can be said about the behavior of the chromosomes during the meiotic phases. Many cells do not show clearly formed chromosomes, and others that do show chromosomes fail to go beyond the metaphase. The chromosomes degenerate early, and the nucleus divides amitotically at the time the cell is undergoing division.

Figure 6, A, shows a metaphase of Prolific with 48 chromosomes on the plate. C and D of Figure 7 are also metaphases of Charles Downing and McCullock, respectively, which have both paired and univalent chromosomes. These three figures serve only to show the number and size of chromosomes. The behavior of most cells was so

variable that no single drawing could be given as typical. Many pollen mother cells seemed to be in the early stages of degeneration, and only in a very small percentage of the cells was there a true meiotic division; there were no cases where cells went into a second-reduction division, so that the least abnormal pollen mother cells made only two pollen grains.

Plate 2, J–Q, is a series of photomicrographs of some of the more regular-behaving cells from representatives of Group 4. J–L are metaphases in Charles Downing showing scattered univalents in cells in which there was some chromosome pairing and more regular plates in cells in which there was no chromosome pairing. M–P show cells of Prolific, Charles Downing, and Cowhorn just before cell division starts. Most of the cells have two nuclei; a few have three unequal-sized nuclei resulting from a single nuclear division. Q shows three pollen groups of two cells each of Cowhorn. Such 2-celled groups are typical of varieties of this group whenever the pollen mother cell has divided. Groups of three cells occasionally arise from cells in which the nucleus has divided into three nuclei, but groups with more than three cells occur very infrequently.

Four other varieties—Maggie Murphy, Carman No. 1, King of the Roses, and Burbank—were included in the 1928 studies. Detailed observations are lacking, but a general study was made which was sufficient to show that they should not be included in either of the more regular groups, 1 or 2.

POLLEN TETRADS

Much has been said in the preceding section of the production of pollen grains from a single pollen mother cell. Normally it is expected to find a group of four cells produced from a single pollen mother cell. To this group has been given the name "pollen tetrad." If, however, only two cells are produced, the name tetrad hardly can be applied even if modified by the term "irregular."

A study of the number of cells in a group produced by a single pollen mother cell gives a fairly reliable picture of the prevalence of irregularities in the reduction division. If there is a predominance of 4-celled groups, the variety is regular; if there is a predominance of other than 4-celled groups, the variety is irregular.

In Table 2 are shown for each variety the prevalent type of reduction division, the number of pollen groups with four cells, the number with more or less than four, and the character of the mature pollen. The figures for the character of the mature pollen were taken from Stout and Clark (*18*) or calculated from observations made by the junior writer.

From Table 2 it can be seen that, with the exception of Prince Albert, those forms classed in Group 1 on the basis of chromosome behavior during pollen formation produce from 75 to 100 per cent 4-cell pollen tetrads and are placed in either class 1 or 2 of the four mature pollen classes described by Stout and Clark (*18*). It is difficult to explain why the mature pollen is poorer in Prince Albert than in others of this group.

Varieties of Group 2 are seen to give between 25 and 75 per cent 4-cell pollen tetrads and have been put in either class 2 or 3 according to the character of their mature pollen. In Group 3 there is very

little production of 4-cell tetrads, and all varieties belong to either class 3 or 4 according to the character of their mature pollen. Group 4 rarely produces a 4-cell tetrad, and all are in class 4 in the character of their mature pollen.

It seems that in a general way one may attribute poor pollen production to some abnormality in the chromosomes at the time of pollen formation. There are few exceptions, and the agreement seems sufficiently close to justify the conclusion that a study of the pollen mother cell development will show the character of the mature pollen of a variety or vice versa.

EFFECT OF ENVIRONMENT

It has often been thought that a variety is more fruitful in one year than in another, and a study of the pollen tetrads in both 1928 and 1929 was undertaken to determine whether the differences in environment in the two years were sufficient to affect the pollen production of a variety.

Twelve varieties of Groups 3 and 4 were studied in both 1928 and 1929, but the production of 4-cell tetrads was under 5 per cent in the 24 counts made. No significant difference could be detected in the counts of any one variety made in two different years. It seems reasonable to assume, therefore, that varieties in these two classes are uniformly irregular in their chromosome behavior and are poor pollen producers under normal but variable growing conditions.

The two varieties of Group 2 in which pollen tetrads were counted in both 1928 and 1929 proved more interesting. Peachblow, for example, in 1928 had over 90 per cent 4-cell tetrads. On the basis of these counts it should have been put in Group 1, but counts from different buds made in 1929 gave an average of about 47 per cent. In Green Mountain the counts made in 1928 show about 43 per cent, while the counts in 1929 average about 5 per cent, varying from 0 to 11 per cent.

In Group 1 two varieties were studied in both 1928 and 1929. Prince Albert, with an 80 per cent production of 4-cell tetrads, was constant over the two years, while McCormick showed 75 per cent 4-cell tetrads in 1928 and only 57 per cent in 1929.

It is apparent from tetrad counts given in Table 2 that the production of 4-cell tetrads varies in varieties that are intermediate between the more fertile and the more sterile classes.

Three of these intermediate varieties—McCormick, Peachblow, and Green Mountain—showed a variable production of 4-cell tetrads in 1928, when samples were taken from buds growing on adjacent plants of the same variety. The counts are not in sufficiently close agreement to ascribe the variability to random sampling alone. Some factor or factors have affected the chromosome behavior during the meiotic phases of pollen formation. The environment in which a plant grows is often sufficiently variable to cause noticeable differences in growth of adjacent plants. The position of a bud in a flower cluster may be favorable or unfavorable, causing considerable variation in the development of individual buds. One is tempted to ascribe the variability in the production of normal pollen of different buds of a progeny to varying environmental conditions.

The growth conditions of two different seasons are not likely to be identical. A study of the production of normal tetrads in the

above-mentioned three varieties was made in 1929, and a variation similar to that of 1928 was found when samples from different buds of the same progeny were compared, but in addition it was found that the production of 4-cell tetrads in all these varieties was lower than in 1928. The differences were too great to be ascribed to random sampling and suggested that growth conditions in 1929 must have been more unfavorable for normal chromosome behavior at the time of pollen formation than in the preceding year.

An examination of weather charts, kindly lent by the superintendent of the Aroostook Farm, showed a difference in the rainfall of the two years, during the week preceding the collection of material, that may have been sufficient to change the growth conditions of potatoes. In 1928 there was ample rain—1.29 inches for the week preceding the collection from McCormick and 1.44 inches for the week preceding the collection from Peachblow and Green Mountain. In 1929, for the same period, there was only 0.8 inch preceding the collection from Green Mountain and no rain at all preceding the collection from Peachblow and McCormick.

That deficiency in soil moisture might be responsible for the more irregular meiotic behavior of 1929 is only suggested. It is the only climatic factor showing a wide difference between the two years, but an extensive study would be required before it could be stated definitely to what extent specific environmental factors modify chromosome behavior during gametic formation.

It seems apparent that in different varieties of potatoes the chromosome complement does not show the harmony observed in wild species. This lack of harmony is shown in varying degree in the varieties listed in Table 2. So pronounced is it in a large percentage of the varieties that there is little or no normal pollen mother-cell development. In a few varieties the chromosomes seem to be more in harmony, and an appreciable number of normal tetrads are produced. This harmony, however, seems easily disturbed, and a comparison of material from different buds collected either in the same year or in different years shows a variability too great to be ascribed to random sampling and suggests that variable growth conditions have affected the chromosome behavior. In a very few varieties the chromosome complement seems to be in harmony and the meiotic behavior is almost as regular as that found in material from wild species.

DISCUSSION

It is apparent from this study that polyploidy is prevalent in the potato and some of its near relatives. Forms have been described with 12, 18, 24, and 36 as their haploid chromosome number. A survey of cytological literature shows that it is unusual to find a species represented by both a diploid and a tetraploid form. The diploid representatives of *Solanum tuberosum* include a small group known as the yellow-fleshed varieties of South America. In them it was found that the haploid chromosome number is 12, while the other 37 cultivated varieties examined in this study all have 24 as their haploid chromosome number. Fukuda (*5*) made a similar study of the chromosomes in 40 varieties of potato and states that the haploid chromosome number for each of them is 24.

Earlier investigators, i. e., Němec (*14*), Martins Mano (*12*), Müller (*13*), and Lutman (*11*) describe varieties of *Solanum tuberosum* with chromosome numbers of about 36. More recent studies by Levitsky and Benetskaja (*10*) and the author referred to in the preceding paragraph seem to make these lower counts unlikely. Levitsky finds in addition to the normal 48 somatic chromosomes that there is a tendency for chromosomes to fragment, which gives the impression that the chromosome number is above 48 in some cells.

The universal presence of the tetraploid chromosome number in our North American cultivated varieties of potatoes precludes the use of chromosome counts as a means of identifying potato varieties. This double chromosome number, however, may be significant from an evolutionary standpoint. It is now generally believed that a form with the basic chromosome number is not as far removed from the ancestral types as polyploid forms. If we consider this to be the case in *Solanum tuberosum*, we must place the yellow-fleshed varieties near the ancestral type. The regular behavior of the chromosomes during the reduction divisions certainly indicates for them a much more stable ancestry than that suggested by the nonharmonious behavior of chromosomes in the tetraploid varieties.

The presence of irregular meioses in potatoes during pollen formation was referred to by Young (*23*), who says that occasionally the homotypic division is omitted. Fukuda (*5*) considers the chief cause of pollen sterility to be due to the abnormal meiotic behavior of the developing pollen mother cells. Stow (*19*), who saw from the work of the two preceding authors the possibility that external conditions might be the cause of irregular pollen mother-cell development, subjected developing buds to extremes of heat and cold. He found that varieties normally behaving regularly will behave irregularly when the temperature is appreciably increased or decreased. Stow's conclusions, however, are hardly applicable to varieties that behave irregularly under the usual growth conditions.

The pollen development in tetraploid potatoes seems to the writers to be disturbed by inherent factors that make the production of normal tetrads exceptional in a large percentage of the varieties studied by the writers. A detailed examination of the types of pollen tetrads produced by three of the more fertile varieties suggests that, in addition to their inherent factors, environment may modify the chromosome behavior during meioses, which in turn determine the character of the pollen tetrad and eventually the amount of viable pollen produced by a variety. A comparison of material collected in 1928 and 1929 showed that in both years the variation in buds from different plants was large, which may be explained by differences in growth conditions of the plant or in the position of the buds on an individual plant. A comparison of the results for the two years indicates that the conditions in 1928 were more favorable for pollen formation than in 1929.

Although environment may modify somewhat the chromosome behavior during the reduction divisions of the developing pollen mother cells, the writers agree with Fukuda (*5*) that the lack of harmony of the chromosome complement of tetraploid varieties of potatoes is the major cause for the failure to produce, in potatoes, normal 4-cell pollen tetrads.

The writers, for convenience and because the irregularities in pollen mother-cell development show characteristic differences, have divided their material into four groups based on the predominance of some characteristic irregularity.

There is first the variable amount of chromosome pairing. Failure of some of the 48 chromosomes to pair could be found in all cultivated varieties; in some, however, it was much more prevalent than in others. Varieties in which the only noticeable irregularity in chromosome behavior during meioses is the failure of some chromosomes to pair, produce a fair amount of viable pollen and are considered our most fruitful varieties.

Combined with the irregularity in the pairing of the chromosomes there may be one or all of the three following unusual abnormalities: (1) Absence of a second reduction division; (2) absence of any tendency for chromosomes to pair; (3) early degeneration of chromosomes, followed by an amitotic division.

Fukuda (*5*) described very similar irregularities in his discussion of the chromosome behavior of potato varieties, although he has grouped the irregularities differently from the writers. Irregular phenomena are so varied and numerous in many of the pollen mother-cell preparations that it would require a long study to interpret and describe them all. The writers have described only the more prevalent irregularities that seemed characteristic of any particular variety or that facilitated obtaining an accurate chromosome determination.

The chromosome behavior of tetraploid potato varieties is frequently sufficiently characteristic to enable one to distinguish by a cytological study varieties in the more fertile classes from those of the sterile class, but this character would be of little service in distinguishing varieties of the same group.

What the writers have found concerning the production of viable pollen only confirms what potato breeders have known for some time. Only a few varieties are useful as pollen parents in potato crosses. The writers have, however, traced one step farther back the cause of nonfruitfulness in potatoes. It is due to the absence of viable pollen, which in turn is due to an abnormal chromosome behavior at the time of pollen formation.

Many of the recent studies of polyploid species suggest explanations for the abnormal chromosome behavior so prevalent in tetraploid potato varieties. But perhaps the recent study of Karpechenko (*8*) best fits the conditions described in this study. In an investigation of the chromosome number and behavior of *Raphanus sativus* L. and *Brassica oleracea* L. he found that both species have the basic chromosome number 9 and that their chromosome behavior is regular. F_1 hybrids of these two species had the same chromosome number as the parents, but the chromosome complement was so inharmonious that no chromosome pairing occurred and pollen development was very irregular, giving gametes with varying chromosome numbers. F_2 plants were found to have from 27 to 78 somatic chromosomes, and the meiotic behavior during pollen formation was varied and strikingly abnormal. Karpechenko's material illustrates well the origin of polyploid forms from hybrids as due to the characteristic irregular chromosome behavior of a hybrid in which the chromosome complement is made up of two distinctly different sets.

In the potato the conditions are reversed. One presumes that nature has been the hybridizer and that tetraploid potatoes represent the outcome. Karpechenko (*8*) points out that if his plants were to propagate apogametically polyploid forms would be established. In potatoes a form becomes established through asexual reproduction, so that a perfectly sterile form may be as long-lived as a more fertile one.

All of our tetraploid potato varieties have a chromosome behavior that suggests a mixed ancestry. If one were to construct a chart showing the possible descent of our cultivated potatoes, it would begin with two or more wild Solanum species having the basic chromosome number 12. From the hybrid progeny of these species have arisen, just as from Karpechenko's hybrid, new forms with double the number of chromosomes, whose hybrid character has been preserved through long periods by asexual reproduction.

The hypothesis of the hybrid origin of polyploid cultivated potatoes is strengthened by the existence of a group of closely related polyploid wild forms. Three of these—*Solanum commersonii*, *S. cardiophyllum* f. *coyoacanum*, and a hybrid resembling *S. maglia* referred to by Clark (*2*)—have a chromosome behavior that strongly suggests that they are natural hybrids and not regular-behaving species. The number and behavior of the chromosomes seem to show that considerable natural crossing occurs among wild species in their native habitat.

Irregular behavior has been duplicated in an artificially produced hybrid of *Solanum fendleri* × *S. chacoense* in which a 24-chromosome set or two 12-chromosome sets are combined with a 12-chromosome set. The irregular meiotic behavior in the developing pollen mother cell is typical of a hybrid produced when plants with different chromosome numbers are crossed.

The heterozygous condition of practically all of our potato varieties for many of their most prominent characters has also been regarded by some as an indication of a hybrid origin. On the other hand it is pointed out by Fukuda (*5*) and by Wittmack (*22*) that the shape of the flower parts, which is an important character in the differentiation of species, has not changed since the time of the earliest descriptions of the potato, over three centuries ago. The constancy of the floral characters is also confirmed by the observations of the junior writer, which are based on the progenies of a large number of crosses among many varieties. This, however, is not a serious obstacle to the theory of a multiple origin, as it is possible that the original progenitors of the present species may have possessed the same type of calyx and corolla and yet have differed in many other characters. If more than one type of calyx and corolla were involved in the early ancestry, it would be possible for the dominant type, especially if linked with desirable economic characters which would insure its preservation, to be carried along for several generations by the usual methods of potato breeding, which consist for the most part in selecting and propagating vegetatively the first or occasionally the second generation of crosses between highly heterozygous parents. So far as the writers are aware, no taxonomic studies have been made of pure lines of potatoes which have been carried through several inbred generations. While in the yellow-fleshed varieties included in these studies the calyx and corolla are of the same general type as those of the

white-fleshed varieties, the differences in the cytological behavior, the number of chromosomes, and other characteristics suggest the probability that these two groups are not of the same immediate ancestry.

Cultivated potatoes have been subjected to a long period of asexual reproduction. This allows the preservation of a herbaceous form that would immediately be lost if it depended on seed reproduction. Has asexual reproduction been the cause of the degeneration of the gametic cells? One of the first plants in which abnormal meioses were described was *Hemerocallis fulva* (Juel (*6*)), a plant that has long been subjected to asexual reproduction, but it is still in doubt whether asexual reproduction has been the cause of abnormalities, since here also they may have been due to the hybrid ancestry of the plant. No authentic case seems to be on record of a regular-behaving species whose fruitfulness has been affected by subjection to long asexual reproduction. On the other hand, very many of our cultivated plants that are propagated asexually often show considerable tendency to be nonfruitful; but they are plants in which hybridization has probably occurred, and the origin of sterility well may be attributed to incompatible chromosome combinations which have been perpetuated by continued asexual propagation.

The investigations of Krantz and Hutchins (*9*) in potato breeding indicate at the present time that a potato variety becomes more uniform after three or four generations of self-fertilization. This effect of inbreeding may be taken as a further demonstration that the domesticated potato has retained the characteristic irregularities of inharmonious chromosome complements simply because it has been propagated asexually. Sexual generations provide an opportunity for gametic selection to eliminate the unbalanced chromosome combinations typical of hybrids between species, and thus result in the production of stable forms.

The conclusions of the writers are at variance with those of Fukuda (*5*), who considers the domesticated potato to have arisen from a single species. It seems more probable that the cultivated potato has a very mixed ancestry, as held by Juzepechuk and Bukasov (*7*), and that the ancestors of the 24-chromosome varieties of *Solanum tuberosum* are to be found not in a single wild species but in two or more.[3]

SUMMARY

This paper presents a study of the number and meiotic behavior of chromosomes in tuber-bearing forms of Solanum.

Ten wild species of potato were found to have 12, 18, 24, or 36 as their haploid chromosome number. Two species, *Solanum commersonii* and *S. cardiophyllum* f. *coyoacanum*, with 18 as their haploid chromosome number, have meiotic irregularities in their pollen

[3] After the manuscript of the present paper was completed, two papers by Rybin (*15*, *16*) were received that are in agreement with several of the conclusions drawn from a study of the material available to the writers. Rybin is of the opinion that all the European and North American commercial varieties of potato probably have 48 as their somatic chromosome number. He finds forms of wild potatoes with 24, 36, 48, 60, and 72 somatic chromosomes. The triploid forms were found to have irregular reduction divisions similar to those described by the writers, and the pentaploid forms were known either to be hybrids or to have characters that suggested a hybrid ancestry. He finds among the local cultivated potato varieties of Central America and South America forms with 24, 36, and 48 chromosomes. His study of the morphological and cytological characters of the triploid forms has led him to conclude that they are hybrids which have either originated from a cross of cultivated species having 24 and 48 chromosomes, respectively, or descended from 36-chromosome wild forms. In his cytological data he gives strong support to the hypothesis that cultivated potatoes have a polyphyletic origin.

mother cells similar to those found in a known F_1 hybrid of *S. fendleri* × *S. chacoense*, suggesting that these two wild species are natural hybrids.

Three cultivated varieties of *Solanum tuberosum* grown in South America have 12 as their haploid chromosome number.

Thirty-seven cultivated varieties of *Solanum tuberosum* grown in the United States have 24 as their haploid chromosome number.

The meiotic behavior of the 24-chromosome *Solanum tuberosum* varies from regular in a few to extremely irregular in many of the varieties.

Only the few varieties with a regular chromosome behavior produce an appreciable amount of pollen; varieties with an irregular chromosome behavior produce practically no pollen. Unfruitfulness in potatoes would therefore seem to be due to abnormal chromosome behavior at the time of pollen formation.

The chromosome behavior of a few selected individuals seemed to be affected to some degree by environmental changes.

The number and behavior of the chromosomes in our cultivated potato varieties suggest that they have a mixed ancestry and that the ancestors are to be found not in a single wild species but in two or more.

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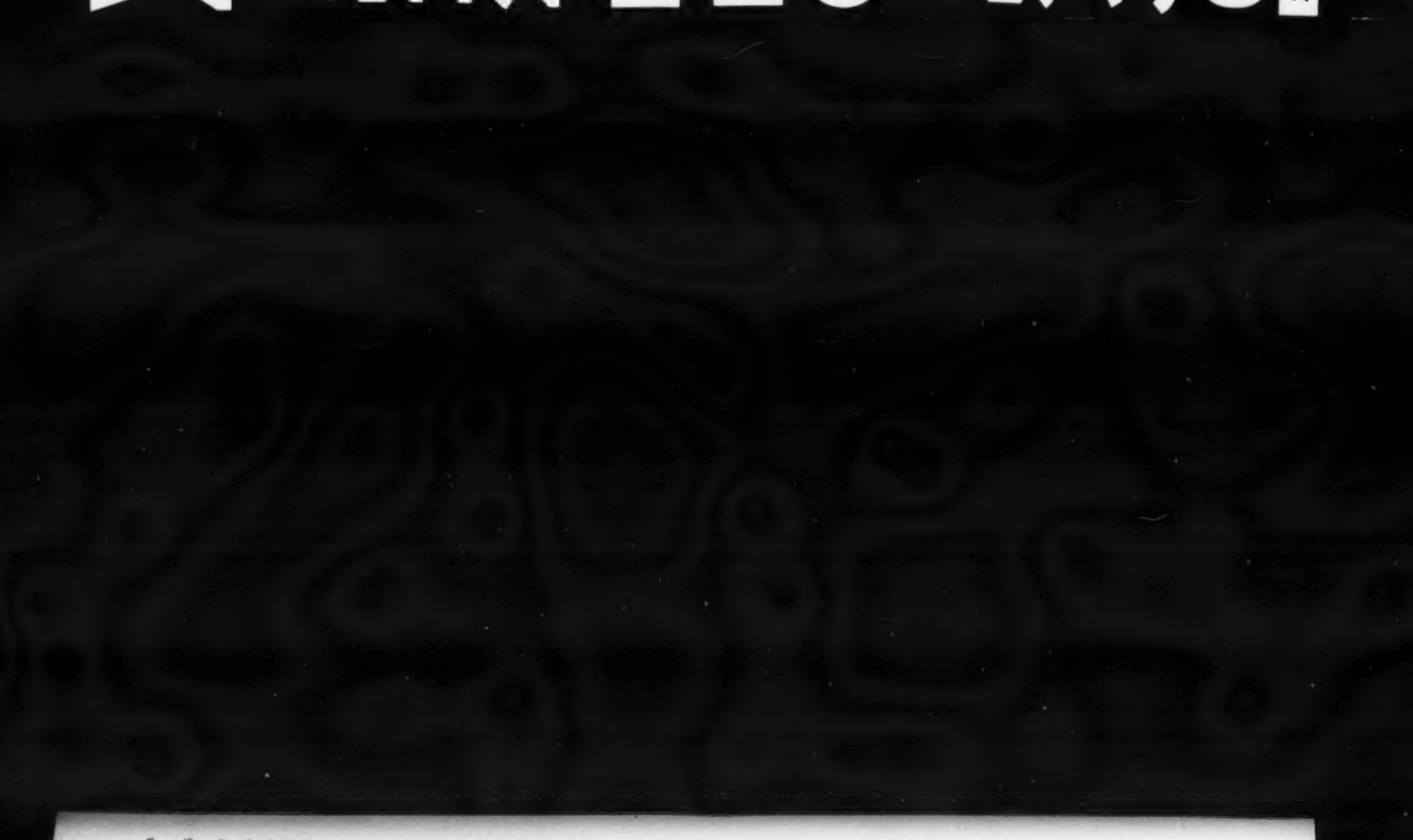
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